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ORIGINAL ARTICLE

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**Diversity and association of phenotypic and metabolomic traits in the close model  
grasses *Brachypodium distachyon*, *B. stacei* and *B. hybridum***

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Running Title: Phenotypic and metabolomic study of annual *Brachypodium* species

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24 *Background and Aims* Morphological traits in combination with metabolite fingerprinting  
25 were used to investigate inter and intra species diversity within the model annual grasses *B.*  
26 *distachyon*, *B. stacei* and *B. hybridum*.

27 *Methods* Phenotypic variation of 15 morphological characters and 2219 nominal mass ( $m/z$ )  
28 signals generated using Flow Infusion Electrospray ionisation - Mass Spectrometry (FIE-MS)  
29 were evaluated in individuals from 174 wild populations and 6 inbred lines and 12 lines of the  
30 three species, respectively. Basic statistics and multivariate Principal Component Analysis  
31 (PCA) and Discriminant Analysis (DA) were used to differentiate inter- and intraspecific  
32 variability of the two types of variables, and their association was assayed with *rcorr*.

33 *Key results* Basic statistics and ANOVA detected eight phenotypic characters [(stomata) leaf  
34 guard cell length, pollen grain length, (plant) height, second leaf width, inflorescence length,  
35 number of spikelets per inflorescence, lemma length, awn length] and 434 tentatively  
36 annotated metabolite signals that significantly discriminated the three species. Three  
37 phenotypic traits (pollen grain length, spikelet length, number of flowers per inflorescence)  
38 might be genetically fixed. The three species showed different metabolomic profiles. DA  
39 significantly discriminated the three taxa with both, morphometric and metabolome traits and  
40 the intraspecific phenotypic diversity within *B. distachyon* and *B. stacei*. The populations of  
41 *B. hybridum* were considerably less differentiated.

42 *Conclusions* Highly explanatory metabolite signals together with morphological characters  
43 revealed concordant patterns of differentiation of the three taxa. Intraspecific phenotypic  
44 diversity was observed between northern and southern Iberian populations of *B. distachyon*  
45 and between E Mediterranean – SW Asian and W Mediterranean populations of *B. stacei*.  
46 Significant association was found for pollen grain length and lemma length and 10 and 6  
47 metabolomic signals, respectively. These results would guide the selection of new germplasm  
48 lines of the three model grasses in on-going GWAS experiments.

49 **Key words:** association studies, *Brachypodium distachyon*, *Brachypodium stacei*,  
50 *Brachypodium hybridum*, metabolite fingerprinting, phenotypic traits, statistic analyses  
51

## INTRODUCTION

Wild plants and, to a lesser extent, cultivated crop species exhibit large phenotypic and metabolomic diversity as a consequence of their individuals' responses to developmental growth conditions and adaptation to environmental factors (Fiehn, 2002). Characterization of phenotypic and metabolomic traits, and their biological dynamics, have become a major line of research in the new era of 'omics' features (Fiehn, 2001, Fiehn, 2002, Hall et al., 2002), due to their importance for Genome-Wide-Association-Studies (GWAS; Matsuda et al., 2015). Thus, phenomic and high resolution metabolomic approaches have been used in conjunction with genomic, transcriptomic and proteomic data to detect and map genes, regulatory sequences and epigenomic regulators in the genomes, and to unravel the plastic and biochemical variation of individuals under different intrinsic and extrinsic scenarios. Phenotypic and metabolomic responses of plants to different development stages and biotic-abiotic conditions have been investigated in crop species, such as cereals (wheat, barley, rice), tomato and potato, and in some model plants, like the dicot *Arabidopsis thaliana* (Allwood et al., 2006). However, these studies are less developed in *Brachypodium distachyon*, the annual temperate grass selected as model plant for the monocots (Draper et al., 2001, IBI, 2010).

Over the past decade, *Brachypodium distachyon* has emerged as one of the preeminent model plant species, with tremendous genetic, molecular, and genomic resources (IBI, 2010, Mur et al., 2011, Catalán et al., 2014, Gordon et al., 2014). Its flagship plant genome now serves as an anchor for genomic studies across the temperate Pooideae grasses and monocots (Lyons and Scholthof, 2015). Its small genome size, compact genome (e. g. low levels of repetitive DNA), diverse ecological tolerances, ready propagation under controlled growth conditions, and considerable existing molecular and genomic resources make this plant an excellent candidate for addressing fundamental questions in comparative genomics and ecological studies and for its transference to cereal and biofuel crops (Catalán et al., 2014).

The analysis of the intraspecific diversity in *B. distachyon* is under way through the nuclear and organellar resequencing of 54 diverse natural accessions (Gordon et al., 2014; and unpublished data). Phenotypic and metabolomics studies conducted to date in *B. distachyon* have mostly focused on relevant agricultural traits, such as plant height, biomass, flowering time, seed size, seed production and vernalization requirements (Draper et al., 2001, Opanowicz et al., 2008, Filiz et al., 2009, Vogel et al., 2009), and stress-tolerance related metabolites (Allwood et al., 2006, Parker et al., 2008, Parker et al., 2009, Pasquet et al., 2014, Onda et al., 2015, Shi et al., 2015).

Recently, the demonstration that the model plant was not one but three species (Catalán et al., 2012) opened the way to a thoroughly comparative genomic study of this diploid-polyploid complex, which include the model grass plant *B. distachyon* and its close allies *B. stacei* and *B. hybridum*, which show, respectively,  $2n=10$ , 20 and 30 chromosomes (Catalán et al., 2012, 2014). These three cytotypes were previously attributed to different ploidy levels of the same taxon *B. distachyon* s. l. (Robertson, 1981); however, phylogenetic, cytogenetic and phenotypic analyses demonstrated that they should be treated as different species. They consist of two diploids, each with a different chromosome base number [*B. distachyon* ( $x=5$ ,  $2n=10$ ); *B. stacei* ( $x=10$ ,  $2n=20$ )], and their derived allotetraploid *B. hybridum* ( $x=5+10$ ,  $2n=30$ ). Phylogenetic analyses indicated that the more basally-diverging *B. stacei* and the more recently evolved *B. distachyon* emerged from two independent lineages, confirming their contribution as genome donors of *B. hybridum* (Catalán et al., 2012, 2014).

Regarding their broad phenotypic and ecological features, individuals of *B. distachyon* have overall small stature, require vernalization and are distributed at higher elevations, those of *B. stacei* are morphologically tall, do not require vernalisation and grow mostly in coastlands or at lower altitudes, and those of *B. hybridum* tend to be physically large,

generally lack vernalisation requirements and grow in intermediate altitudinal places (Opanowicz et al., 2008, Catalán et al., 2012, López-Alvarez et al., 2015). Statistical analysis of morphometric traits showed that five characters (stomata leaf guard cell length, pollen grain length, upper glume length, lemma length, and awn length) significantly discriminated between the three species when they were grown under controlled greenhouse conditions (12 inbred lines) and showed stability in 24 additional wild populations (Catalán et al., 2012). However, representation of the three circum-Mediterranean species was biased towards the more exhaustively sampled populations of the western Mediterranean region. This was particularly critical for *B. stacei*, a species that was only known from its type locality at the time of its description (Catalán et al., 2012), and for which no other statistically analyzed phenotypic data has been provided to date despite its known distribution in other native circum-Mediterranean localities (López-Alvarez et al., 2012, 2015).

Recently, López-Alvarez *et al.*, (2012) provided an alternative and reliable genetic method to differentiate the individuals of the three species using a DNA barcoding system using three loci, the plastid *trnLF* region and the nuclear multicopy ribosomal ITS spacer and the low copy Gigantea (GI) gene, which successfully discriminated between the three species. Interestingly, this study also demonstrated the existence of different bidirectional crosses that likely gave rise to the allotetraploid *B. hybridum*. This was confirmed through the analysis of the maternally inherited plastid haplotypes in this species; the majority of the surveyed *B. hybridum* individuals had inherited a maternal *B. stacei*-like plastome but some of the accessions showed a maternal *B. distachyon*-like plastome (López-Alvarez et al., 2012). The recurrent, polyphyletic and polytopic origin of this allotetraploid species was supported by ITS and GI evolutionary analyses, which revealed distinct relationships of the *B. hybridum* sequences to different parental geographic haplotypic groups, though all the studied hybrids correspond to what is considered to be the same allopolyploid species (López-Alvarez et al.,

2012, Catalán et al., 2016b). Nonetheless, the potential phenotypic differences between these two types of reciprocal hybrids have not been investigated to date.

Metabolomics is considered to represent the ultimate level of ‘omic’ analysis, facilitating the testing of many biological hypotheses based on the statistical support obtained from high-throughput processing of samples (Allwood et al., 2006, Draper et al., 2013), and references therein). Different metabolomics studies have been used to identify different metabolic pathways, wild type and mutant accessions, and sensitive *vs* tolerant lines to biotic and abiotic stresses in plants (Allwood et al., 2006). In *Brachypodium distachyon*, metabolomic analyses have shown to be useful for identifying the main metabolites produced in responses to fungal diseases (Allwood et al., 2006, Pasquet et al., 2014), to temperature-salinity (Onda et al., 2015) and drought (Shi et al., 2015) stresses, and the dynamics of host/pathogen interactions (Parker et al., 2008, 2009).

Non-targeted metabolite fingerprinting is a technique designed to provide a relatively comprehensive view of the metabolome that can be used to test competing hypotheses in organisms (Draper et al., 2013) where prominent changes in the metabolome are revealed without specifically identifying individual metabolites. This method, originally based on Fourier-transform infrared (FT-IR) spectroscopy, was successfully applied to discriminate between control and abiotically stressed lines (Johnson et al., 2003) and responses between tolerant and sensitive lines to fungal attack in plants (Allwood et al., 2006). An additional advantage of metabolite fingerprinting is that the robustness of results can be tested by validation of the analysis using different biological replicates as training and test sets, respectively (Draper et al., 2013). Nonetheless, the combined use of ‘first-pass’ metabolic fingerprinting and ultra-high accurate mass metabolite profiling, based on direct-injection ESI-MS, which facilitates the identification of particular metabolites, allow for a deeper understanding of the biochemical processes involved in the investigated case studies.



Currently, the increased accuracy of mass spectrometry actually promotes ESI-MS from a merely fingerprinting technique to a metabolite profiling tool, which combined with MS/MS capabilities improves the structural information of the molecules (Draper et al., 2013). A preliminary metabolic fingerprinting analysis conducted in different accessions of diploid *B. distachyon* found different metabolic profiles related to geography (Opanowicz et al., 2008). However, no metabolomic study has been performed to date in *B. stacei* and *B. hybridum*.

The new evolutionary and genomic findings within the *B. distachyon* s. l. complex taxa have set the stage for high definition research of the unusual genomic diversity between and within the species of this complex. The nuclear and organellar genomes of *B. stacei* and *B. hybridum* are being sequenced and will serve, together with *B. distachyon*, as a model system for investigating the origins and consequences of the speciation and polyploidization events (Catalán et al., 2014; and unp. data) that might parallel those of economically important cereals (e. g., wheats; Marcussen et al., 2014). Despite these advances, the phenotypic and metabolomics studies of the three species of the complex are still incomplete or have not been performed yet. Given the importance of these investigations for future GWAS analysis, the objectives of this study are, 1) to analyse the phenotypic variation of a large representation of individuals (and populations) of *B. distachyon*, *B. stacei* and *B. hybridum* collected across their respective native distributions in the circumMediterranean region and in some non-native sites; 2) to test the value of potentially informative morphological traits to discriminate between the three species and within geographic ranges and biological origins (*B. hybridum* only) of each species; 3) to comparatively analyse the metabolite profiles of native accessions of *B. distachyon*, *B. stacei* and *B. hybridum*; 4) to test the value of potentially informative metabolomic traits to discriminate between the three species; 5) to analyse the potential association of phenotypic and metabolomic variation between the three species of the *B. distachyon* s. l. complex.

## MATERIALS AND METHODS

### *Sampling*

An enlarged sampling was performed in order to increase the representation of populations and individuals of the three *B. distachyon* s. l. complex species in their respective native circumMediterranean regions and in few non-native areas of *B. hybridum* (Fig. 1, Table S1). The aim was to cover as much intraspecific phenetic and environmental variability as possible, considering that geographic distribution and environmental variability might affect phenetic variability. Intraspecific phenetic diversity for each of the three species under study has been evidenced in previous studies (Vogel et al., 2009, Catalán et al., 2016b). Additionally, an environmental niche model study indicated that *B. distachyon*, *B. stacei* and *B. hybridum* show overlapping but different environmental niches in their native circumMediterranean region where each species presents a different range of variation for different sets of environmental parameters (López-Alvarez et al., 2015). Also, intraspecific environmental variability has been recorded in the three taxa (Manzaneda et al., 2012, Manzaneda et al., 2015, Shiposha et al., 2016). A total of 1050 individuals, of which 870 were newly collected ones, from 174 wild populations of *B. distachyon* (227 individuals, 44 populations), *B. stacei* (146 ind., 30 pop.) and *B. hybridum* (497 ind., 100 pop.), plus the 180 individual samples from the 6 inbred lines of *B. distachyon* [Bd21(type), ABR1]; *B. stacei* [ABR114, type], and *B. hybridum* [ABR113 (type), ABR110, ABR117] employed in the study of Catalan et al., (2012), were used in the phenotypic analysis. The new samples were collected in the field or were obtained from herbaria and germplasm banks. Seed from the inbred lines (several generations of selfing) and from new wild germplasm collections (first generation individuals mostly derived from different mother plants) were germinated and grown under standard greenhouse conditions following Catalán et al., (2012). All the studied materials were analysed phenotypically when they reached maturity (e. g., flowering and

fruiting stages). The geographic origins and nature [wild (W), herbaria (H), seed bank (S) or inbred (I) plants] of all the studied samples are indicated in supplementary Table S1. The taxonomic identity of all the new samples was corroborated through DAPI-staining chromosome counting and/or DNA barcoding methods following the procedures indicated in López-Alvarez *et al.*, (2012). Herbarium vouchers of the newly collected materials have been deposited in the JACA and Unizar (University of Zaragoza) herbaria.

### *Phenotypic analysis*

Phenotypic analysis was performed using the same 15 potentially informative morphoanatomical characters that were employed to separate and to identify the three species of the *B. distachyon* s. l. complex in a previous study (Catalán *et al.*, 2012). Twelve of the characters were quantitative [(Plant) Height (H); Second Leaf Length (SLL); Second Leaf Width (SLW); (Stomata) Leaf Guard Cell Length (LGCL); Inflorescence Length (IL); Spikelet Length (total, without awns; SLa); Spikelet Length (from base to 4<sup>th</sup> lemma, without awns; SLb); Upper Glume Length (UGL); Lemma Length (LL); Awn Length (AL); Caryopsis Length (CL); Pollen Grain Length (PGL)], and three were discrete characters [Number of Nodes of Tallest Culm (NNTC); Number of Spikelets per Inflorescence (NSI); Number of Flowers per Inflorescence (NFI)] (Tables 1, S2). Macromorphological characters were measured with a hard ruler under a dissecting microscope. Microanatomical characters (LGCL; PGL) were measured under a microscope using an ocular micrometer. For measurements of the stomata leaf guard cell length, abaxial epidermises were peeled off from dried leaves which were pre-treated in a 90% lactic acid solution for 8 hours. Up to ten individuals (specimens) per population were used for assessment of morphological characters. When possible, five measurements were taken for each character in each individual and the corresponding averaged values were used in the analyses. Statistical analyses were performed

separately for wild vs inbred + wild individuals (data from inbred individuals was retrieved from Catalán *et al.*, (2012). Discriminant analysis was also performed for wild vs inbred + wild individuals (see below).

Simple statistic descriptors of the intra-species and inter-species phenetic diversity (mean, range, standard deviation, box plots of median, range and percentiles) were calculated from the data. The analysis of the inter-species response variables was estimated through one way ANOVA chi-square tests or through nonparametric Kruskal–Wallis tests when the variables complied or not, respectively, with requirements of normality (this was tested through Kolmogorov-Smirnov (K-S) tests; see Table S2). Multiple pairwise comparisons of means were based on Tukey’s tests for groups with unequal samples sizes. Inter-species response variables were evaluated through a multicollinearity analysis in order to determine which characters were correlated with each other in the common data set and in each of the species data sets; a matrix of Spearman correlation coefficients was obtained by averaging the values from each individual in each case. Comparative analysis of variables from wild populations vs inbred lines was performed using averaged values and through pairwise Mann-Whitney (U) tests. In all cases significant tests were performed for the null hypothesis (H0) of  $\mu I = \mu W$ , where  $\mu I$  and  $\mu W$  correspond to averaged trait values of inbred lines and wild populations, respectively. All the statistical analyses were conducted with the software SPSS v. 15.

#### *Multivariate analysis of phenotypic traits*

Multivariate Principal Component Analysis (PCA) of the 15 variables was performed to examine the structure of the taxa, to assess if the observed groupings were consistent with the taxonomic circumscriptions proposed for the three species of the *Brachypodium distachyon* s. l. complex, and to evaluate the level of covariation in variables. Averaged

values of the 15 morphoanatomical characters were estimated for the 174 wild populations and 6 inbred lines of *B. distachyon*, *B. stacei* and *B. hybridum*. The contribution of each character to the coordinate axes that accumulate the highest percentages of variance was calculated by covariance and variance matrices of the samples with respect to the new axes using PAST v. 2.17 (Hammer et al., 2001). Further PCAs were conducted in each of the *B. distachyon*, *B. stacei* and *B. hybridum* subgroup samples following the procedure of the previous search. These independent analyses would allow estimation of the intraspecific substructure of the taxa and calculation of the participation of different morphological characters to the separation of intraspecific groups within each species.

A classification Discriminant Analysis (DA, cross-validation) was conducted with all the variables (15) and samples (174 wild populations and 6 inbred lines) to determine the highest probability membership group of the samples (Legendre and Legendre, 1998). The reference samples for each species's group were the respective type specimens (*B. distachyon*: Bd21; *B. stacei*: ABR114; *B. hybridum*: ABR113). The identification of the more discriminating variables was done by means of Fisher's coefficient (Fisher, 1936, Anderson, 1996) at the significant threshold value of 0.05. The posterior probability of classification of each sample and the Wilks' Lambda value of each discriminant function were calculated. A Wilks' Lambda value closer to zero indicated a better discrimination between the predefined groups. DA was run in SPSS v. 15. Further DAs were also performed following the same procedure indicated above within each of the *B. distachyon*, *B. stacei*, *B. hybridum* groups aiming to classify the samples into intraspecific groups, using as references for each subgroup flag samples from the intraspecific PCA analyses (see Results).

#### *Metabolomic analysis*

Sampling for metabolomic analysis was performed in a subset of populations from the

three studied species (Table S1). Individuals from 12 populations or inbred lines (5 of *B. distachyon*: 115F, 160F, 162F, 480F, 484F; 3 of *B. stacei*: 114F, 129F, 485F, and 4 of *B. hybridum*: 137F, 176F, 260F, 333F), representing 12 different ecotypes, were grown under standard greenhouse conditions and used in the metabolomic study. A hierarchical metabolomics approach was used to identify and annotate metabolites discriminating the three species. Non-targeted nominal mass metabolite fingerprinting was followed by in depth data mining and ultra-high accurate mass metabolite profiling of explanatory  $m/z$  bins. Data base searches,  $MS^n$  fragmentation analysis and comparison with available standards aided signal annotation at different Metabolomics Standard Initiative (MSI) levels of identification (Sumner et al., 2007).

Metabolite fingerprinting was performed using Flow Infusion Electrospray ionisation Mass Spectrometry (FIE-MS). For this, sample extraction process and mass spectrometric analysis was done following Parker *et al.*, (2009) and Draper *et al.*, (2013). This process involved the use of a single-phase extraction solvent (chloroform: methanol: water, 1: 2.5: 1, v: v: v) optimized for recovery of a wide range of metabolites, offering relatively comprehensive coverage of the metabolome. Nominal mass FIE-MS analysis was performed using a Linear Trap Quadrupole (LTQ) mass analyzer (Finnigan LTQ; Thermo-Finnigan, San Jose, CA), which generated metabolite fingerprints in both positive and negative ionisation mode. Ion intensities were detected in the scan range between  $m/z$  50 and 1150 that was subdivided in four small mass ranges for better signal acquisition (low range,  $m/z$  15-200; high1 range,  $m/z$  180-620; high2 range,  $m/z$  600-880, and high3 range,  $m/z$  860-1150), and raw data dimensionality was reduced by electronically extracting signals with  $\pm 0.1$  Da mass accuracy. Mass spectra were combined in a single intensity matrix (runs x  $m/z$  ratios) for each ion mode. Data from intensity matrix was log-transformed and normalized to the total ion count (TIC) before further statistical analysis. Initially, data mining and feature selection was performed

using Random Forest in R package *FIEms-pro* as reported previously (Team, 2010, Enot et al., 2008).

Ionization products ( $m/z$ ) identified after data mining were annotated by searching accurate  $m/z$  through the MZedDB database (Aberystwyth University) (<http://maltese.dbs.aber.ac.uk:8888/hrmet/index.html>) at  $< 5$  ppm mass accuracy. Accurate masses from species representative extracted samples were acquired using Exactive LC-MS system (Thermo-Scientific) operating in flow-infusion mode. As several overlapping solutions predicting the presence of different metabolites were often possible, the most likely combination of ions putatively identifying a specific metabolite were confirmed by comparing their  $MS^n$  fragmentation patterns with available standards.  $MS^n$  fragmentation was carried out on Finnigan LTQ (Thermo-Finnigan, San Jose, CA) using ion trap, full MS mode by using a normalized collision energy of 30.0 V. Samples were injected at a flow rate of 3.0  $\mu\text{L} / \text{min}$  with activation (Q) of 0.250 and activation time of 30.0 ms. 30 scans were used to acquire fragmentation data with an isolation width of 1.0  $m/z$ .

#### *Statistical and multivariate analysis of metabolomic traits*

Metabolomic data were subjected to simple statistical analysis of the inter-specific diversity (mean, range, standard deviation, box plots of median, range, intervals) and the response variables were estimated through one-way ANOVA chi-square tests and through nonparametric Kruskal-Wallis for parametric and non-parametric variables, respectively, to determine which metabolites were significantly different between the *B. distachyon*, *B. stacei* and *B. hybridum* ecotypes. Only metabolites that were annotated at different MSI levels of identification (see Supplementary data) were used in the multivariate analysis. DA was conducted with the metabolomic data; this consisted of a supervised projection method where discrimination between groups was based on the spatial classification of ecotypic samples in

2D projections using a priori knowledge of species projections. The Fisher's coefficient ( $p = 0.05$ ) was used to identify the more discriminating variables, and the posterior probability of classification of each sample and the Wilks' Lambda value of each discriminant function were also calculated. All statistical analyses were conducted in SPSS v. 15.

### *Associated phenotypic and metabolomic variation*

The potential association of phenotypic and metabolic variation between the three species of the *B. distachyon* s. l. complex was estimated through correlation analysis using Pearson coefficients. Class means (at ecotype level) of both the 15 phenotypic measurements and the 434 metabolic variables ( $m/z$  intensity values) were used for correlation analysis as individual phenotypic measurements could not be attributed to the metabolomic data. Correlation analysis was performed using *rcorr* in R package *Hmisc* and resulting  $p$ -values were corrected by Bonferroni method.

## RESULTS

### *Interspecific phenotypic variation*

Statistical descriptors and box and whisker plots (Table 1, Fig. 2) summarized the inter and intraspecific phenotypic diversity detected by the 15 analysed morphological characters across the studied wild populations of the three species. Significant differences ( $P < 0.001$ ) were found in 13 variables for different combinations of species (Table 1): eight characters discriminate all three species from each other (LGCL, PGL, H, SLW, IL, NSI, LL, AL) (Fig 2a), four characters discriminate between *B. distachyon* vs. *B. stacei* and *B. hybridum* (SLL, SLa, SLb, CL) (Fig. 2b), and one character discriminate *B. hybridum* from the diploid species (NNTC) (Fig. 2c). Only two characters did not significantly discriminate the species (NFI, UGL) (Table 1; Fig. 2d). The individuals of *B. hybridum* showed mean values significantly



higher for three characters, stomata guard cell length (LGCL;  $\bar{x}=29.3 \pm 3.3\mu\text{m}$ ), pollen grain length (PGL;  $\bar{x}=38.7 \pm 0.121\mu\text{m}$ ) and number of nodes of tallest culm (NNTC;  $\bar{x}=5.7\pm 2.4$ ), than individuals from the diploid species (*B. distachyon*,  $\bar{x}=22.5$ ;  $\bar{x}=30.4$ ;  $\bar{x}=4.0$ ; *B. stacei*,  $\bar{x}=25.2$ ,  $\bar{x}=33.9$ ,  $\bar{x}=4.4$ ), while individuals of *B. stacei* showed mean values significantly higher for six characters, plant height (H;  $\bar{x}=43.6 \pm 17.4$ ), second leaf width (SLW;  $\bar{x}=2.7\pm 0.9$ ), inflorescence length (IL;  $\bar{x}=5.3\pm 1.5$ ), lemma length (LL;  $\bar{x}=9.4\pm 1.3$ ), awn length (AL;  $\bar{x}=12.4\pm 2.7$ ), and number of spikelets per inflorescence (NSI;  $\bar{x}=3.3\pm 0.8$ ) than individuals of *B. hybridum* and *B. distachyon* (Table 1; Fig. 2a-c). Individuals of *B. distachyon* were characterized by smaller and shorter leaf, inflorescence, spikelet, lemma, awn and caryopsis than those of the other species; they also showed few nodes in the stem and less spikelets and flowers per inflorescence (Table 1; Fig 2). Individuals of *B. hybridum* showed intermediate dimensions between those of individuals of its parental species in seven characters (H, SLW, IL; NSI, LL, AL, CL) (Table 1; Fig. 2).

When mean value data obtained from the analysis of wild individuals (W; wild vs wild) were compared with those obtained from inbred individuals (I; inbred vs inbred) (Table 2; W-I Mann-Whitney test: wild vs inbred) three characters (PGL, SLA, NFI) did not show significant differences between the two groups in the three species, two (H, NSI) in *B. stacei* and *B. hybridum*, and two (LGCL, AL), four (IL, SLB, UGL, LL) and one (SLE) in *B. distachyon*, *B. stacei* and *B. hybridum*, respectively. From the three common stable traits in both data sets, PGL discriminated between all three species and SLA between *B. distachyon* and *B. stacei* or *B. hybridum*; NFI did not discriminate between species (Table 2). LGCL, LL and AL also discriminated between all three species, and SLL and SLB between *B. distachyon* and *B. stacei* or *B. hybridum* in both data sets but with different means in W and I for some species, SLW between the three species in W (but only between *B. distachyon* and *B. stacei* or *B. hybridum* in I), and CL between *B. distachyon* and *B. stacei* or *B. hybridum* in

W (and between *B. stacei* and *B. distachyon* or *B. hybridum* in I) (Table 2). Two traits that significantly discriminated between the three species within the inbred lines (I), discriminated only between *B. hybridum* and *B. distachyon* or *B. stacei* (NNTC), or did not discriminate between them (UGL) in the larger data set of wild individuals (W) (Table 2). The Spearman correlation analysis conducted with the phenotypic traits studied in the wild individuals of *B. distachyon*, *B. stacei* and *B. hybridum* revealed significant correlations ( $p < 0.001$ ) between several characters [SLA/SLB (0.89), IL/NSI (0.75), H/IL (0.70), SLW-SLL (0.69), H/SLL (0.66), H/NSI (0.65), IL/SLA (0.65), NFI/SLA (0.62), LL/SLA (0.59), NFI/SLB (0.59), LL/SLB (0.56), NNTC/SLL (0.56), LGCL/PGL (0.54), H/SLW (0.53) and H/NNTC (0.52)] (Table S3).

The PCA revealed that 93.3% of total variation of the 15 analysed characters could be explained by the first three principal components which accumulated 79.2%, 10.4% and 3.6% of the variance, respectively (Table S4, Fig. 3). Three characters were identified as the most important contributors to the positive and negative extremes of the first components of the PCA. Plant height (0.98), pollen grain length (0.88) and stomata leaf guard cell length (-0.61) showed the highest contributions to the first, second and third components, respectively (Table S3). The impact of these components on the hierarchical structure of the three species was visualized in a 2D PCA plot (Fig. 3). Population samples of the three species overlapped in the two-dimensional space created by the first two components. The highest overlap was observed between the minimum convex polygons of *B. stacei* and *B. hybridum* (Fig. 3).

The DA conducted over all 174 wild populations of *B. distachyon*, *B. stacei* and *B. hybridum* using the data from the 15 analysed phenotypic traits separated the three species in the two-dimensional plot constructed with the two functions (Fig. 4a). *B. distachyon* clustered separately from *B. stacei* and *B. hybridum* along the first axis of the plot, which explained 68.2% of the total variance, whereas the last two species separated along the second axis,

which explained 31.8% of the variance. Catalán *et al.* (2012) also found similar discrimination among the three species when the DA was performed only with individuals from the 6 inbred lines, though sampling of *B. stacei* was reduced to the type specimen (ABR114). Equivalent results were obtained when DA was performed with the 6 inbred lines added to the 174 wild populations (Fig. 4b); the three species showed similar clustering patterns in the 2D plot which had functions 1 and 2 explaining 66.8 and 33.2% of the total variance, respectively. All *B. distachyon* populations were correctly classified (100%) both including (46 samples) and excluding (44) the inbred lines (Table 3). However, only 25 (83%) of 30 wild populations of *B. stacei* were correctly classified (with the 5 remaining samples B891, B921, B621, Bra286 and B100-H141 classified as *B. hybridum* (17%)), and only 25 (81%) of 31 wild + inbred samples were correctly assigned (with 6 remaining samples classified either as *B. hybridum* (5, 17%) or *B. distachyon* (BGE044241, 3%)). Most samples of *B. hybridum* were correctly classified in both analysis (92%); 92 out of 100 wild populations, and 95 out of 103 wild + inbred samples, were correctly assigned, with the 8 remaining samples classified either as *B. stacei* (5: B124, 333F, BGE044239, BGE044247, Mog; 5%) or *B. distachyon* (3: BGE044243, B741, Bra299; 3%) in both cases (Table 3). Up to ten and five phenotypic characters showed strong correlations to the first and second canonical discriminant functions, respectively (Table S5), with Wilk's Lambda values of 0.12 and 0.44 ( $p < 0.001$ ), respectively. Of those, three (LGCL, CL and PGL) showed the highest contributions to function 1 and one (IL) to function 2 in the wild populations and the combined inbred lines and wild populations data sets [LGCL (0.54, 0.56), CL (0.47, 0.46) and PGL (0.42, 0.44); IL (0.52, 0.53)] (Table S5).

#### *Intraspecific phenotypic variation*

The DAs conducted at the intraspecific level in *B. distachyon*, *B. stacei* and *B.*

*hybridum* using the 15 phenotypic traits showed significant differences for some traits and different geographical groupings of populations in one and another studied species. In *B. distachyon*, the two-dimensional plot constructed with the first two discriminant functions that explained 79.7% and 10.8% of the total variance indicated the separation of four groups of Iberian populations (Fig 5a). Northeastern Iberian populations (Aragón, Navarra, Lleida) clustered in the upper left area of the plot, Northwestern Iberian populations (Valladolid, Palencia) and southern Iberian populations (Andalucía) plus one additional population from southern Spain (Cadiz) in the lower left and middle areas, and central Iberian populations (Albacete, Cuenca, Madrid, Rioja) in the upper right area. The characters that showed higher correlations to functions 1 and 2 were H (0.56) and UGL (0.66), AL (0.46) and NNTC (0.46) ( $p < 0.001$ ), respectively, with Wilk's Lambda values of 0.012 ( $p < 0.001$ ) and 0.15 ( $p = 0.058$ ) (Table S6), respectively. The height of the plant (H) was significantly different between the central ( $\bar{x} = 42.2^a$ ) and the northwestern ( $\bar{x} = 32.2^b$ ) groups, which in turn showed taller individuals than those of the northeastern ( $\bar{x} = 18.6^c$ ), and southern ( $\bar{x} = 13.8^c$ ) Iberian groups (Table 4). The length of the upper glume (UGL) significantly separated the southern ( $\bar{x} = 4.3^b$ ) populations from the others ( $\bar{x} = 6.4^a$ ), the number of nodes in the tallest culm (NNTC) the northeastern populations ( $\bar{x} = 2.7^b$ ) from the rest ( $\bar{x} = 6.4^a$ ), the length of the awn (AL) the northeastern ( $\bar{x} = 11.2^a$ ) from the southern ( $\bar{x} = 9.5^b$ ) populations, and the number of spikelets per inflorescence (NSI) the central ( $\bar{x} = 3.0^a$ ) from the northeastern and southern ( $\bar{x} = 1.8^b$ ) populations (Table 4).

In *B. stacei* the 2D plot constructed with the first two discriminant functions that accumulated 79.4% and 11.4% of the total variance classified three groups of circumMediterranean populations (Fig 5b). The Iranian populations clustered at the upper right area of the plot, and the Balearic populations at the lower right area; they were clearly separated from the other populations along function 1. Populations from Israel clustered close

to populations from Almeria (S Spain) in the upper middle area of the plot, those from the Canary Islands in the middle central area, those from southern Spain and Mahgreb (Tunisia) in the leftmost central area, and one population from Greece in the lowest left area (Fig. 5b). The phenotypic traits that showed higher correlations to functions 1 and 2 were IL (-0.11) and SLB (0.18) and SLA (0.17) ( $p < 0.001$ ) (Table S7), respectively, with Wilk's Lambda values of 0 ( $p < 0.001$ ) in both cases. The pollen grain length (PGL) significantly discriminated the Iranian populations, which showed longer pollen grains ( $\bar{x} = 47.2^a$ ) than the rest ( $\bar{x} = 32.3^b$ ) (Table 4). IL, NNTC and SLL traits separated southern Spain ( $\bar{x} = 3.7^b$ ;  $\bar{x} = 2.8^b$ ;  $\bar{x} = 9.2$ ) from Balearic Island ( $\bar{x} = 6.1^a$ ;  $\bar{x} = 6.2^a$ ;  $\bar{x} = 4.3$ ) populations, NNTC and SLL southern Spain from the Israel ( $\bar{x} = 5.8^a$ ;  $\bar{x} = 5$ ) populations, and SLL southern Spain from Iran ( $\bar{x} = 4.5$ ) populations; the remaining populations showed intermediate measurements for those traits ( $\bar{x} = 5.2^{ab}$ ;  $\bar{x} = 4.6^{ab}$ ;  $\bar{x} = 6.9$ ) (Table 4).

In *B. hybridum* the first two discriminant functions of the two-dimensional DA plot that explained 42.7% and 31.4% of the total variance did not show a clear-cut geographical clustering of circumMediterranean populations though they separated two main groups along the first axis (Fig 5c). Populations from northeastern Spain and the Balearic islands clustered in the left area of the plot whereas those from the Middle-East, the Mediterranean basin, southern Spain and the Canary Islands clustered predominantly on the right area. The most influential characters were H (0.51), SLW (0.44) and CL (0.40), and NNTC (0.57), UGL (0.48), and NFI (0.42), which were highly correlated to the first and second functions ( $p < 0.001$ ), respectively (Table S8), with Wilk's lambda values of 0.02 and 0.08 ( $p < 0.001$ ), respectively. The height of the plant separated the taller Mediterranean and Canary Islands individuals ( $\bar{x} = 47^a$ ;  $\bar{x} = 45.8^a$ ) from the shorter Balearic Islands and northern Spain individuals ( $\bar{x} = 18.1^c$ ;  $\bar{x} = 21.5^c$ ), whereas individuals from other regions (southern Spain  $\bar{x} = 36.7^b$ ; Middle East  $\bar{x} = 35.3^b$ ) showed intermediate statures (Table 4). Caryopsis length (CL)

and width of the second leaf (SLW) differentiated the individuals from Middle East and Canary Islands ( $\bar{x}=7.4^a$ ;  $\bar{x}=2.7^a$ ) from those of northern Spain and Balearic Islands ( $\bar{x}=6.2^c$ ;  $\bar{x}=1.7^c$ ), whereas the Mediterranean and southern Spain individuals showed intermediate values ( $\bar{x}=6.6^b$ ;  $2.4^{ab,b}$ ) (Table 4). The number of nodes in the tallest culm was higher in Mediterranean and southern Spain individuals ( $\bar{x}=6.8^a$ ;  $\bar{x}=6.2^a$  respectively; Table 4) with respect to those of Middle East ( $\bar{x}=5.2^b$ ) and Balearic Island, Canary Islands and northern Spain ( $\bar{x}=3.8^c$ ;  $\bar{x}=3.3^c$ ;  $\bar{x}=2.8^c$ ) individuals.

#### *Interspecific metabolomic variation*

Metabolite fingerprinting analysis resulted in a total of 2219 nominal  $m/z$  in combined positive and negative ionisation datasets in the 12 studied ecotypes of *B. distachyon*, *B. stacei* and *B. hybridum*. However, only 693 metabolite signals showed significant differences between species based on Random Forest feature selection, and of these only 434 nominal  $m/z$  signals could be further annotated using targeted accurate mass  $m/z$  search through MZedDB database (Table S9). Where more than one  $m/z$  signals resulted in the annotation of same metabolite e.g. due to various adducts formation, the most likely adduct was selected based on either, the most abundant adduct or by giving preference for  $[M+H]^{1+}$  and  $[M+K]^{1+}$  adduct in positive ion mode and  $[M-H]^{1-}$  adduct in negative ion mode over the other adducts. The other duplicate adducts were however kept in the annotation list of 434  $m/z$  signals (in plain text rather than bold) (Table S9) as they give aided confidence in the annotation of  $m/z$  signals. These  $m/z$  signals with duplicate annotations were however later removed from the DA, details of which are provided below. Some of the annotation results were confirmed by comparing  $MS^n$  fragmentation patterns of  $m/z$  signals with that of available standard's (see Table S10 for the list of annotated  $m/z$  signals).

Statistical descriptors and box and whisker plots indicated that 434 fingerprint  $m/z$

signals were able to significantly discriminate between the three species (Tables S11, S12; Fig. 6). Of these, several positive (e. g., p214.09, p147.09, p182, p296.18, p139.9, p816.54, p235.09, p381.18) (Fig. 6a) and negative (e. g., n281.18, n385.18, n346.09, n315.18, n163, n203.09, n135, n179.09) (Fig. 6b)  $m/z$  discriminated the ecotypes of *B. distachyon*, *B. stacei* and *B. hybridum* between each other (Table S12).

The DAs conducted over the 12 ecotypes of *B. distachyon*, *B. stacei* and *B. hybridum* using the data from the 434 (positive 217, and negative 217) metabolomic traits discriminate the three species in the two-dimensional plots formed by the two functions (Table S11; Fig. 7). Removing  $m/z$  signals of same annotations showing different adducts does not alter DA classification results (Tables S9, S11; Fig. S1). The five ecotypes of *B. distachyon* clustered separately from those of *B. stacei* and *B. hybridum* along the first axis of the plot, which explained 84.7% (positive data set) or 80% (negative data set) of the total variance, whereas the ecotypes of the last two species separated respectively along the opposite extremes of the second axis, which explained 20% (positive data set) or 15.3% (negative data set) of the variance. Both positive and negative metabolites were significantly correlated with the first (Wilk's Lambda = 0;  $p < 0.001$ ) and second (Wilk's Lambda = 0.01 (positive data set), 0.02 (negative data set);  $p < 0.001$ ) functions of their respective analysis though their correlation values were usually low (Table S11). The ecotypes of each species were correctly classified to their respective groups in all cases (100%).

#### *Association of phenotypic traits and metabolites*

The Pearson correlation analysis between phenotypic traits and 434 fingerprint  $m/z$  signals indicated that the highest significant Bonferroni corrected  $p$ -values  $< 0.05$  correlations were for pollen grain length (PGL) and lemma length (LL) (Table 5) to discriminate the three species. PGL showed significant correlations with 10 metabolite signals (hydroxybutyrate,

threonate, shikimate,  $^{13}\text{C}$  isotope of shikimate, quinate,  $^{13}\text{C}$  isotope of quinate, sinapate, sedoheptulose 7-phosphate, ADP-glucose, PG(18:1(11Z)/22:6 (4Z,7Z,10Z,13Z,16Z,19Z)), and LL showed significant correlations with six metabolites (*O*-Phosphohomoserine and lipids tentatively assigned as PC(16:0/18:2(2Z,4Z)), SQDG(16:0/16:1(11Z)), PC(18:3(8E,10E,12E)/18:3(8E,10E,12E)) (PC(36:6)), PC(18:0/18:3(9Z,12Z,15Z)/0:0), PG(18:0/20:3(5Z,8Z,11Z))) (Table 5 and Fig. S2).

## DISCUSSION

*Phenotypic differentiation is shaped both taxonomically and geographically within the B. distachyon s. l. complex species*

The large phenotypic analysis conducted on 1050 individuals from 174 wild populations and 6 inbred lines of *B. distachyon*, *B. stacei* and *B. hybridum* has provided a wealth of data and strong statistical evidence for disentangling the intraspecific phenotypic plasticity and the interspecific differentiation of the three species, once considered cryptic taxa (Garvin et al., 2008, Catalán et al., 2012, López-Alvarez et al., 2012). The considerably increased sampling size of this study with respect to the pioneer work of Catalán *et al.*, (2012) has allowed to also increase the number of significantly discriminating traits between the species from five to eight (Table 1, Fig. 2). To the five characters (stomata leaf guard cell length, pollen grain length, upper glume length, lemma length, awn length) found previously Catalán *et al.*, (2012) to significantly discriminate between the three taxa when inbred lines were grown under controlled greenhouse conditions, the present study adds four more characters (plant height, second leaf width, inflorescence length, number of spikelets per inflorescence) and discards one (upper glume length) for discriminating between wild individuals of the three species. Exhaustive analysis shows that despite an increase of intraspecific phenotypic variability within each species (Tables 1, 2; Fig. 2) when compared



to Catalán *et al.*, (2012), the number of discriminant taxonomic traits also raises (from the same 15 original characters) as a consequence of the more robust statistical inference and the underlying evolutionary phenotypic differentiation of the three members of the complex (Catalán *et al.*, 2012, 2014).

This study has also shown geographically-diverse phenotypic data for the poorly known species *B. stacei* (Tables 1, 2, S1; Fig. 2). This taxon emerges as the tallest plant and with some of the largest features (inflorescence, no. spikelets/inflorescence, lemma and awn) of the three species of the complex, oversizing the measurements of the allotetraploid *B. hybridum* for those traits (Table 1, Fig. 1). Additionally, this study confirms previous findings of Catalán *et al.*, (2012) like smaller stature and shorter features (12 out of the 15 studied traits) of *B. distachyon* with respect to its two congeners. The considerable phenotypic gap observed between the two diploid species *B. stacei* and *B. distachyon* (Table 1; Fig. 2) could be a consequence of their distinct evolutionary origins, large divergence and genomic expressions (Catalán *et al.*, 2012, 2014). The allotetraploid *B. hybridum* shows significantly larger measurements for two non-endoreduplicating plant cell types, stomata guard cell length and pollen grain length, than its two diploid progenitors (Table 1). Correlation between increasing ploidy level and larger pollen grain and stomata guard cell sizes have been reported in other pooids, like *Lolium* (Speckmann *et al.*, 1965), *Bromus* (Tan and Dunn, 1973), and *Dactylis* (Bretagnolle and Lumaret, 1995). These traits could be also used as proxies to differentiate *B. hybridum* from its parents.

The use of morphological traits is considered to be limited, because most of the traits are multigenic, quantitative characters that could be influenced by environmental conditions, plant age, phenological stage or cultivation conditions (Smykal *et al.*, 2008). However, presented data support the stability of the phenotypic characters in natural populations when compared to the propagated inbred lines (Table 3). Most of the studied characters show the

same discriminant value to separate between wild, cultivated, or wild and cultivated individuals of *B. distachyon*, *B. stacei* and *B. hybridum* (Tables 3, S5). Furthermore, three of the traits (pollen grain length, spikelet length, number of flowers per inflorescence) have shown to be similarly discriminant among the three species, both in wild and inbred plants. Results of this study suggest that these traits could be genetically fixed and might constitute a valuable tool to separate and identify individuals of the *B. distachyon*, *B. stacei* and *B. hybridum*. However, the precise phenotypic characterization and identification of all individuals is not always possible, as demonstrated by the failure to correctly classify a few individuals of *B. stacei* and *B. hybridum*, erroneously assigned to other species (Table 3, Figs. 4a,b). Because the taxonomic identity of those individuals was confirmed by chromosome counting and/or DNA barcoding, their diverging phenetic features could have resulted from extreme plasticity or a consequence of the restricted number of phenotypic traits used in the study. By contrast, the low percentages of failures detected (up to 20% in *B. stacei*, 8% in *B. hybridum*, and 0% in *B. distachyon*) suggest that the employed morphological traits are good diagnostic features to differentiate these taxa. In a recent updated taxonomic description of these species, five additional qualitative phenotypic traits have been found useful discriminators: leaf blade color to separate all three species, occasional production of short rhizomes to discriminate *B. stacei* and *B. hybridum*, and leaf blade shape, softness and hairiness to separate *B. stacei* from the other two species (Catalán et al., 2016a).

The analysis of the phenotypic variation within each of the three species of the *B. distachyon* complex has also untapped the organization of their intraspecific diversity (Fig. 5). The geographic phenotypic structure observed between the northern, central and southern Iberian populations of *B. distachyon* (Fig. 5a) might reflect different adaptations to environmental conditions (Manzaneda et al., 2015) and could be also indicative of genotypic differences (López-Álvarez & Catalán, unp. data). Also, the observed differences in plant

height (H), with central and northern Spanish individuals being significantly taller than southern Spanish individuals (Table 4), has been corroborated in independent phenomic studies of Iberian *B. distachyon* inbred lines (E. Pérez-Collazos & J. Finch, pers. comm.). The broader but less congruent geographic structure of *B. stacei* phenotypes (Fig. 5b) suggests the isolation of circumMediterranean-edge Iranian and continental-island Balearic populations *versus* the proximity of largely disjunct Israel-southern Spain (Almeria) and southern Spanish-Canary Islands populations. Long distance dispersals of seeds have been proposed for *B. distachyon* and *B. hybridum* (Vogel et al., 2009) and could also operate in *B. stacei*. The low geographically structured variation within *B. hybridum* detects however a slight differentiation of the Balearic and northern Spanish populations from the rest (Fig. 5c). It is important to stress, however, that the southern Spanish allotetraploid individuals derived from the unusual cross of maternal *B. distachyon* parent and paternal *B. stacei* parent (cf. López-Alvarez et al., 2012) are morphologically close to other Mediterranean individuals derived from the common cross of maternal *B. stacei* and paternal *B. distachyon* (Fig. 5c). In allopolyploid grasses different bidirectional crosses of parental species might originate the same or different hybrid allopolyploid species (e. g., *Aegylops*; Meimberg et al., 2009). However, our data corroborate the idea of a unique speciation event in the origin of *B. hybridum*, even if its individuals could have originated from alternative bidirectional crosses between different ancestral diploid progenitors (Catalán et al., 2016b).

#### *Metabolomic characterization of B. distachyon, B. stacei and B. hybridum and association of phenotypic-metabolite traits*

FIE-MS fingerprinting has provided a preliminary metabolite profile for the three species of the *B. distachyon* complex. The study shows that a large number of metabolites (434) could be used to discriminate between *B. distachyon*, *B. stacei* and *B. hybridum* (Tables

S9-S12; Fig. 6). For example, citrulline content is significantly larger in *B. hybridum* than in its parental species, of which *B. distachyon* shows the lowest concentration (Fig. 6A). Citrulline has been shown to play an important role in transporting and storing nitrogen, and is reported as an important biochemical indicator of plant tolerance to saline and drought stresses (Kawasaki et al., 2000, Kusvuran et al., 2013). In this study, citrulline levels reflect a climatic variation from warmer and more aridic Iberian places (*B. hybridum* ecotypes), through warm but shady places (*B. stacei* ecotypes), to more mesic places (*B. distachyon* ecotypes) (Fig. 1; Catalán et al., 2016a), supporting the different ecological adaptations of the three species' ecotypes to their respective environments. Another example is a phospholipid assigned as phosphatidylcholine(36:6) (PC(36:6)) whose levels were significantly larger in *B. hybridum* than in its parental species (Fig. 6A). Drought stress has been shown to induce changes in the leaf lipid composition by increasing the levels of phosphatidylcholine suggesting specific adaptive alterations in the membrane composition to compromise drought stress tolerance (Vigh et al., 1986, Toumi et al., 2008).

DA analysis indicates not only that each species could be significantly separated from each other (Figs. 7; S1) but that *B. distachyon* falls clearly apart from *B. stacei* and *B. hybridum*, followed by metabolomic differentiation of the later. This pattern parallels what has been observed in the phenotypic analysis (Fig. 4) and in molecular relationships between the species, especially in the plastid genome-based reconstructions (López-Alvarez et al., 2012). Thus, metabolite fingerprinting is concordant with phenotypic analysis within the species *B. distachyon* complex. The Pearson correlation analysis between phenotypic and metabolomics traits indicated a significant association for pollen grain length (PGL) and lemma length (LL) with respectively two distinct groups of metabolites, members of the phenolics biosynthesis and lipids. PGL showed significant correlations with metabolite signals for Shikimate, <sup>13</sup>C Isotope of Shikimate, Sinapate, Quinate, <sup>13</sup>C Isotope of Quinate as

652 well as Sedoheptulose-7-phosphate, ADP-Glucose, and a PG(40:7). LL correlated mainly  
653 with lipids, including phosphatidylcholines (PCs) (Table 5). Since the correlation analysis  
654 applied here is only preliminary and these phenotypic traits are among the best markers to  
655 discriminate the three species, especially PLG is likely genetically fixed (Tables 2, S3), one  
656 has to consider chance: high significant correlations found between metabolites and  
657 phenotypic traits might reflect their statistical value to separate the three species, rather than a  
658 direct association with the sizes of the pollen grain and lemma length. However, there is  
659 evidence that these results indicated a potentially strong link between PGL and lignin /  
660 phenolics biosynthesis, and LL and lipid metabolism. PGL can be associated with phenolics  
661 composition as it provides structural support to pollen grain, because mature angiosperm  
662 pollen grains are covered by three distinct layers of cell walls: (1) an outer exine coating,  
663 composed of a tough, chemically resistant biopolymer sporopollenin, which is interrupted by  
664 openings called apertures; (2) an inner intine coating, made primarily of cellulose, and (3) a  
665 pollen coat, composed of lipids, proteins, pigments, and aromatic compounds that fills the  
666 sculptured cavities of the pollen exine wall (Edlund et al., 2004). The sporopollenin is present  
667 in the spore / pollen walls of all land plants, comprising both aliphatic (unsaturated lipids),  
668 and aromatic (phenolics) components, and is regarded as one of the most recalcitrant  
669 biomacromolecules, providing protection against the harsh terrestrial environments including  
670 a range of abiotic stresses (de Leeuw et al., 2006, Fraser et al., 2012). Results therefore show  
671 that phenolics biosynthesis may be crucial for pollen grain development as they provide  
672 structural components to both sporopollenin and pollen coat formation. An alternative  
673 explanation is that the levels of accumulation of those metabolites in the different species  
674 indicate that some biochemical pathways have or have not been switched on yet. A genome-  
675 wide SNP scan study to identify trait-regulatory genomic loci in chickpeas showed the up-  
676 regulation of a superior gene haplotype correlated with increased transcript expression of

*Ca\_Kabuli\_CesA3* gene in the pollen and pod of high pod/seed number accession, resulting in higher cellulose accumulation for normal pollen and pollen tube growth (Kujur et al., 2015). Environmental triggers like temperature or drought might also influence availability of metabolites for enzymatic systems regulating pollen grain and lemma length, but gaps in our knowledge of how constraints affect plant survival and seed production are being filled only slowly (Ejzmond et al., 2011).

A number of studies have corroborated the differentiation of the three species of the *B. distachyon* complex, like those based on seed protein data (Hammami et al., 2011), phenotypic and cytogenetic traits and nuclear and plastid phylogenetic markers (Catalán et al., 2012), nuclear SSRs (Giraldo et al., 2012), DNA barcoding (López-Alvarez et al., 2012), isozymes (Jaaska, 2014), and Comparative Chromosomes Painting (CCP; Idziak et al., 2011, Betekhtin et al., 2014). This study shows that metabolomics can discriminate the three species. Furthermore, preliminary metabolite discriminant analysis suggests a closer metabolomic affinity of *B. hybridum* to its maternal *B. stacei* parent than to its paternal *B. distachyon* parent for the studied ecotypes (Fig. 7). The closeness of the allotetraploid hybrid to its *B. stacei* parent, supported by both phenotypic and metabolomic data (Figs. 4, 7) is also in agreement with whole genome sequence (Vogel, com. per.) and environmental data; environmental niche model analyses demonstrated a larger niche overlap and niche affinity of the *B. hybridum* niche to that of *B. stacei* than to the *B. distachyon* niche (López-Alvarez et al., 2015). Because phenotypic and metabolomic data reflect the summed effects of genotypic composition and environmentally-mediated gene expression, larger genomic, phenomic and metabolomic analyses should be conducted on higher numbers of replicates and different ecotypes of the three species to identify the allelic variants and regulatory elements responsible for the observed phenotypic and metabolomics profiles at both, species and ecotype levels. The preliminary phenotypic and metabolomic analyses applied in this study

have set the way for future, more exhaustive, GWAS studies.

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## SUPPLEMENTARY INFORMATION

A detailed description of phenotypic and metabolomics variables analyzed in inter and intraspecific statistical studies of *Brachypodium distachyon*, *B. stacei* and *B. hybridum* populations is included as Tables S1 to S12 and Figures S1 and S2 in the supplementary information.

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902 **Table 1.** Statistics of 15 phenotypic traits and significance tests of their mean values analyzed in individuals from 174 wild populations of  
903 *Brachypodium distachyon*, *B. stacei* and *B. hybridum*. Underlined variables are those that significantly discriminate between the three species. N,  
904 number of wild individuals analysed. ANOVA (F; d.f. 2) or Kruskal-Wallis ( $X^2$ ; d.f. 2) tests of variables used for comparisons between species.  
905 Superscripts denote Tukey pairwise comparisons between species; means with the same letter do not differ significantly ( $p < 0.05$ ). See text and  
906 Table S2 for abbreviations of variables.  
907

<b>Species</b>	<b><u>LGCL</u></b> ( $\mu\text{m}$ )	<b><u>PGL</u></b> ( $\mu\text{m}$ )	<b><u>H</u></b> (cm)	<b>NNTC</b>	<b>SLL</b> (cm)	<b><u>SLW</u></b> (mm)	<b><u>IL</u></b> (cm)	<b><u>NSI</u></b>	<b>SLA</b> (cm)	<b>SLB</b> (cm)	<b>NFI</b>	<b>UGL</b> (mm)	<b><u>LL</u></b> (mm)	<b><u>AL</u></b> (mm)	<b>CL</b> (mm)
<b><i>B. distachyon</i></b>															
N	227	211	191	190	171	174	184	184	187	183	188	191	188	190	184
Minimum	17	21	5,5	1	1,1	0,5	0,92	1	0,92	0,5	4	2,2	5,2	6,3	3,8
Maximum	28	42	56	12	8,2	3,5	5	4	2,4	1,82	13	9,1	11	15,2	7,2
<b>Mean</b>	<b>22,5<sup>a</sup></b>	<b>30,4<sup>a</sup></b>	<b>19,8<sup>a</sup></b>	<b>4,0<sup>a</sup></b>	<b>3,0<sup>a</sup></b>	<b>1,7<sup>a</sup></b>	<b>2,3<sup>a</sup></b>	<b>2,1<sup>a</sup></b>	<b>1,6<sup>a</sup></b>	<b>1,1<sup>a</sup></b>	<b>7,9<sup>a</sup></b>	<b>6,0<sup>a</sup></b>	<b>7,2<sup>a</sup></b>	<b>10,7<sup>a</sup></b>	<b>5,5<sup>a</sup></b>
Std. Deviation	2,2	3,8	11,9	2,4	1,5	0,5	0,8	0,8	0,3	0,2	2,1	1,5	0,9	1,9	0,7
Variance	5,0	14,4	141,8	5,9	2,2	0,3	0,6	0,7	0,1	0,1	4,4	2,3	0,8	3,7	0,4
<b><i>B. stacei</i></b>															
N	146	154	86	90	75	82	88	91	90	91	88	90	91	88	123
Minimum	16	22	6,1	1	1,6	1,1	2,3	2	1,3	0,61	4	2,9	6,1	7,5	5,4
Maximum	36	52	76	9	15,1	5	10	5	3,3	2,82	14	8,63	12,6	18,2	8,4
<b>Mean</b>	<b>25,2<sup>b</sup></b>	<b>33,9<sup>b</sup></b>	<b>43,6<sup>c</sup></b>	<b>4,4<sup>a</sup></b>	<b>7,4<sup>b</sup></b>	<b>2,7<sup>c</sup></b>	<b>5,3<sup>c</sup></b>	<b>3,3<sup>c</sup></b>	<b>2,2<sup>b</sup></b>	<b>1,6<sup>b</sup></b>	<b>8,5<sup>a</sup></b>	<b>5,8<sup>a</sup></b>	<b>9,4<sup>c</sup></b>	<b>12,4<sup>c</sup></b>	<b>6,9<sup>b</sup></b>
Std. Deviation	4,4	5,9	17,4	1,7	3,3	0,9	1,5	0,8	0,4	0,4	2,3	1,2	1,3	2,7	0,7
Variance	19,5	35,2	301,7	3,0	11,0	0,7	2,2	0,6	0,1	0,2	5,5	1,4	1,7	7,5	0,5
<b><i>B. hybridum</i></b>															
N	497	518	330	340	320	327	347	350	343	340	343	352	349	349	413
Minimum	20	25	3,5	1	1	0,7	1,2	1	1	0,5	2,33	2,3	3	6	5
Maximum	37	57	78	11	15,2	4,3	8	6	4,1	2,96	16	9,8	12,9	18,9	8,9
<b>Mean</b>	<b>29,3<sup>c</sup></b>	<b>38,7<sup>c</sup></b>	<b>35,9<sup>b</sup></b>	<b>5,7<sup>b</sup></b>	<b>7,5<sup>b</sup></b>	<b>2,4<sup>b</sup></b>	<b>3,5<sup>b</sup></b>	<b>2,7<sup>b</sup></b>	<b>2,1<sup>b</sup></b>	<b>1,5<sup>b</sup></b>	<b>8,1<sup>a</sup></b>	<b>5,9<sup>a</sup></b>	<b>8,9<sup>b</sup></b>	<b>11,6<sup>b</sup></b>	<b>6,7<sup>b</sup></b>
Std. Deviation	3,3	5,6	15,3	2,4	3,3	0,7	1,2	1,1	0,5	0,4	2,8	1,5	1,8	2,7	0,8
Variance	10,8	31,2	235,3	5,5	11,0	0,5	1,4	1,2	0,2	0,2	7,7	2,2	3,3	7,4	0,6
$X^2$	411,6	322,1	164,5	82,1	213,2	127,2	255,9	86,3	194,6	132,2				22,9	
<b>F</b>											1,5	0,6	92,3		208,7
<b>p</b>	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	>0.05	>0.05	<0.001	<0.001	<0.001

908 **Table 2.** Comparative Mann-Whitney (U) test of mean values obtained from individuals of 174 wild populations (see Table 1) vs 6 inbred lines  
909 (data retrieved from Catalán et al. 2012) for the 15 analysed phenotypic characters in *B. distachyon*, *B. stacei* and *B. hybridum*. W- Wild  
910 populations; I- Inbred lines (means with the same letter do not differ significantly ( $p < 0.05$ ) between species after Tukey's (wild populations) and  
911 Mann-Whitney (inbred lines) pairwise comparison tests in each independent data set). Variables that do not differ significantly ( $p < 0.05$ ) between  
912 the two compared data sets (W vs I) for each character and species are underlined.  
913

	LGCL	PGL	H	NNTC	SLL	SLW	IL	NSI	SLA	SLB	NFI	UGL	LL	AL	CL
<b><i>B. distachyon</i></b>															
W	<b>22,5<sup>a</sup></b>	<b>30,4<sup>a</sup></b>	<b>19,8<sup>a</sup></b>	<b>4,0<sup>a</sup></b>	<b>3,0<sup>a</sup></b>	<b>1,7<sup>a</sup></b>	<b>2,3<sup>a</sup></b>	<b>2,1<sup>a</sup></b>	<b>1,6<sup>a</sup></b>	<b>1,1<sup>a</sup></b>	<b>7,9<sup>a</sup></b>	<b>6,0<sup>a</sup></b>	<b>7,2<sup>a</sup></b>	<b>10,7<sup>a</sup></b>	<b>5,5<sup>a</sup></b>
I	23.26 <sup>c</sup>	29.87 <sup>c</sup>	26.13 <sup>b</sup>	3.30 <sup>c</sup>	6.68 <sup>b</sup>	2.84 <sup>b</sup>	3.25 <sup>a</sup>	2.69 <sup>a</sup>	1.63 <sup>b</sup>	1.27 <sup>b</sup>	7.00 <sup>b</sup>	7.23 <sup>b</sup>	8.05 <sup>c</sup>	11.45 <sup>a</sup>	6.75 <sup>a</sup>
U	2820,5	3084,5	425,5	910	79	46,5	559,5	791,5	1196	441,5	832	538,5	425	918,5	110,5
p	<u>0,07</u>	<u>0,61</u>	0,03	0,82	0	0	0	0,03	<u>0,92</u>	0	<u>0,12</u>	0	0	<u>0,12</u>	0
<b><i>B. stacei</i></b>															
W	<b>25,2<sup>b</sup></b>	<b>33,9<sup>b</sup></b>	<b>43,6<sup>c</sup></b>	<b>4,4<sup>a</sup></b>	<b>7,4<sup>b</sup></b>	<b>2,7<sup>c</sup></b>	<b>5,3<sup>c</sup></b>	<b>3,3<sup>c</sup></b>	<b>2,2<sup>b</sup></b>	<b>1,6<sup>b</sup></b>	<b>8,5<sup>a</sup></b>	<b>5,8<sup>a</sup></b>	<b>9,4<sup>c</sup></b>	<b>12,4<sup>c</sup></b>	<b>6,9<sup>b</sup></b>
I	28.22 <sup>b</sup>	32.58 <sup>b</sup>	35.56 <sup>c</sup>	2.94 <sup>b</sup>	11.06 <sup>a</sup>	4.15 <sup>a</sup>	6.12 <sup>b</sup>	2.75 <sup>a</sup>	2.29 <sup>a</sup>	1.61 <sup>a</sup>	9.24 <sup>a</sup>	6.24 <sup>c</sup>	8.91 <sup>b</sup>	7.27 <sup>c</sup>	5.68 <sup>b</sup>
U	3325	2903,5	236,5	333	186	171,5	423	380	901	897	806,5	691,5	758	129	165
p	0	<u>0,94</u>	<u>0,06</u>	0	0	0	<u>0,13</u>	<u>0,07</u>	<u>0,74</u>	<u>0,45</u>	<u>0,36</u>	<u>0,06</u>	<u>0,08</u>	0	0
<b><i>B. hybridum</i></b>															
W	<b>29,3<sup>c</sup></b>	<b>38,7<sup>c</sup></b>	<b>35,9<sup>b</sup></b>	<b>5,7<sup>b</sup></b>	<b>7,5<sup>b</sup></b>	<b>2,4<sup>b</sup></b>	<b>3,5<sup>b</sup></b>	<b>2,7<sup>b</sup></b>	<b>2,1<sup>b</sup></b>	<b>1,5<sup>b</sup></b>	<b>8,1<sup>a</sup></b>	<b>5,9<sup>a</sup></b>	<b>8,9<sup>b</sup></b>	<b>11,6<sup>b</sup></b>	<b>6,7<sup>b</sup></b>
I	33.64 <sup>a</sup>	38.27 <sup>a</sup>	33.09 <sup>a</sup>	3.72 <sup>a</sup>	10.30 <sup>a</sup>	2.75 <sup>b</sup>	3.93 <sup>a</sup>	2.71 <sup>a</sup>	2.22 <sup>a</sup>	1.56 <sup>a</sup>	8.15 <sup>ab</sup>	7.92 <sup>a</sup>	10.49 <sup>a</sup>	9.68 <sup>b</sup>	7.02 <sup>a</sup>
U	5090	14182	1636,5	1551,5	1377,5	2025,5	2528	3619	3321	2401,5	3310,5	919,5	1452	1784,5	8781,5
p	0	<u>0,78</u>	<u>0,58</u>	0	0,01	<u>0,06</u>	0,04	<u>0,9</u>	<u>0,81</u>	0,03	<u>0,79</u>	0	0	0	0,01

**Table 3.** Assignment probabilities of individuals from 174 wild populations (W) and from 6 inbred lines plus 147 wild populations (I+W) of *B. distachyon*, *B. stacei* and *B. hybridum* based on discriminant analysis of 15 phenotypic traits. N, number of populations and populations + inbred lines studied.

Data sets	Samples	N	Predicted Group Membership		
			<i>B. distachyon</i>	<i>B. stacei</i>	<i>B. hybridum</i>
W	<i>B. distachyon</i>	44	44 (100%)	0	0
	<i>B. stacei</i>	30	0	25(83,3%)	5(16,7%)
	<i>B. hybridum</i>	100	3(3%)	5(5%)	92(92%)
I+W	<i>B. distachyon</i>	46	46 (100%)	0	0
	<i>B. stacei</i>	31	1 (3,2%)	25(80,6%)	5 (16,1%)
	<i>B. hybridum</i>	103	3 (3%)	5 (5%)	95 (92%)

919 **Table 4.** ANOVA test of 15 variables used for comparisons among wild individuals from intraspecific groups within each species  
920 (*Brachypodium distachyon*, *B. stacei*, *B. hybridum*). Superscripts denote Least Significant Difference (LSD) pairwise comparisons between  
921 geographic groups within species; means with the same letter do not differ significantly ( $p < 0.05$ ). N, number of individuals analysed. Characters  
922 with significant differences only for some comparisons: *B. distachyon*:\* IL=North-East  $\neq$  South; North-West  $\neq$  South; Cádiz  $\neq$  North-East +  
923 South + Center + North-West. \*\* NSS= North-West  $\neq$  North-East + South; Cádiz  $\neq$  South. *B. stacei*:\* \*\*SLL= South  $\neq$  Balearic Islands, Israel,  
924 Iran

Species	N	LGCL	PGL	H	NNTC	SLL	SLW	IL	NSS	SLA	SLB	NFS	UGL	LL	AL	CL
<b><i>B. distachyon</i></b>									*	**						
North-East	23	23,2	30,1	13,8 <sup>c</sup>	2,7 <sup>b</sup>	2,5 <sup>c</sup>	1,8	2,3	2,1	1,7	1,1	8,5 <sup>b</sup>	6,5 <sup>a</sup>	7,3	11,2 <sup>ab</sup>	5,3
South	8	23,0	28,6	18,6 <sup>c</sup>	5,5 <sup>a</sup>	3,1 <sup>bc</sup>	1,5	1,8	1,6	1,5	1,1	6,7 <sup>c</sup>	4,3 <sup>b</sup>	7,1	9,5 <sup>c</sup>	5,8
Center	3	22,1	28,5	32,2 <sup>b</sup>	7,3 <sup>a</sup>	4,3 <sup>ab</sup>	2,1	2,6	2,4	1,6	1,0	7,8 <sup>bc</sup>	6,1 <sup>a</sup>	7,0	10,0 <sup>bc</sup>	5,0
North-West	6	21,4	30,9	42,3 <sup>a</sup>	6,5 <sup>a</sup>	5,5 <sup>a</sup>	1,9	2,8	3,0	1,5	1,2	7,3 <sup>bc</sup>	6,7 <sup>a</sup>	7,4	10,7 <sup>bc</sup>	5,6
Cádiz	1	21,4	34,8	36,4 <sup>ab</sup>	4,0 <sup>ab</sup>	2,6 <sup>bc</sup>	1,8	4,3	3,0	2,0	1,6	12,5 <sup>a</sup>	6,7 <sup>a</sup>	7,6	14,0 <sup>a</sup>	6,5
F		1,2	1,1	32,2	12,9	8,6	1,4	5,2	4,9	1,6	2,0	3,7	7,1	0,5	3,5	2,2
p		>0.05	>0.05	<0.001	<0.001	<0.001	>0.05	<0.05	<0.05	>0.05	>0.05	<0.05	<0.001	>0.05	<0.05	>0.05
<b><i>B. stacei</i></b>									***							
Almeria	4	26,9	33,2 <sup>b</sup>	45,7 <sup>ab</sup>	3,6 <sup>ab</sup>	6,4	2,8	5,5 <sup>ab</sup>	3,1	2,7	1,9	10,0	5,0	10,1	12,1	6,7
South	6	22,3	30,1 <sup>b</sup>	55,9 <sup>a</sup>	6,2 <sup>a</sup>	9,3	2,9	6,1 <sup>a</sup>	3,6	2,1	1,4	7,2	6,3	9,5	14,0	6,8
Canary Islands	4	25,0	31,3 <sup>b</sup>	49,1 <sup>a</sup>	4,0 <sup>ab</sup>	6,1	2,4	5,3 <sup>ab</sup>	3,5	2,1	1,4	8,6	5,8	9,2	10,5	6,8
Balearic Islands	4	27,6	36,6 <sup>b</sup>	19,0 <sup>c</sup>	2,8 <sup>b</sup>	4,4	2,3	3,7 <sup>b</sup>	2,5	2,0	1,5	9,5	5,8	8,6	11,5	6,4
Israel	6	25,7	35,8 <sup>b</sup>	42,3 <sup>ab</sup>	5,8 <sup>a</sup>	5,0	2,5	5,3 <sup>ab</sup>	3,3	2,2	1,7	8,7	6,4	10,2	15,1	6,9
Iran	3	28,5	47,2 <sup>a</sup>	27,7 <sup>bc</sup>	3,6 <sup>ab</sup>	4,5	2,3	4,1 <sup>ab</sup>	2,3	2,6	2,1	11,6	5,6	9,3	14,3	7,7
Tunisia	1	28,6	30,9 <sup>b</sup>	42,0 <sup>abc</sup>	4,0 <sup>ab</sup>	4,2	1,2	5,4 <sup>ab</sup>	3,0	2,2	1,7	10,0	5,7	7,4	11,5	6,4
Greece	1	19,7	28,6 <sup>b</sup>	65,0 <sup>a</sup>	5,8 <sup>ab</sup>	11,0	2,8	6,0 <sup>ab</sup>	4,3	1,8	0,9	7,8	5,2	10,4	14,2	6,1
F		1,8	8,1	17,0	4,6	5,4	1,4	4,1	3,7	1,7	2,0	1,9	1,0	1,9	2,0	2,3
p		>0.05	<0.001	<0.001	<0.05	<0.001	>0.05	<0.05	<0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
<b><i>B. hybridum</i></b>																
North Spain	7	30,3	35,5 <sup>b</sup>	21,5 <sup>c</sup>	2,8 <sup>c</sup>	3,5 <sup>c</sup>	1,8 <sup>c</sup>	4,0 <sup>b</sup>	2,9 <sup>abc</sup>	2,2 <sup>b</sup>	1,3 <sup>b</sup>	9,9 <sup>a</sup>	7,2 <sup>ab</sup>	8,1 <sup>c</sup>	12,5 <sup>ab</sup>	6,3 <sup>bc</sup>
Canary Islands	10	28,8	44,0 <sup>a</sup>	45,8 <sup>a</sup>	3,4 <sup>c</sup>	6,6 <sup>b</sup>	2,7 <sup>ab</sup>	5,2 <sup>a</sup>	3,2 <sup>ab</sup>	2,8 <sup>a</sup>	1,9 <sup>a</sup>	11,1 <sup>a</sup>	7,6 <sup>a</sup>	10,2 <sup>ab</sup>	11,8 <sup>bc</sup>	7,4 <sup>a</sup>
South Spain	23	29,5	36,4 <sup>b</sup>	36,7 <sup>b</sup>	6,2 <sup>a</sup>	7,9 <sup>ab</sup>	2,3 <sup>b</sup>	3,3 <sup>c</sup>	2,7 <sup>bc</sup>	2,1 <sup>b</sup>	1,5 <sup>b</sup>	7,3 <sup>b</sup>	5,4 <sup>c</sup>	9,4 <sup>b</sup>	11,6 <sup>bc</sup>	6,7 <sup>b</sup>
Balearic Islands	15	28,5	38,8 <sup>b</sup>	18,2 <sup>c</sup>	3,8 <sup>c</sup>	3,5 <sup>c</sup>	1,6 <sup>c</sup>	2,7 <sup>d</sup>	1,9 <sup>d</sup>	2,1 <sup>b</sup>	1,4 <sup>b</sup>	9,6 <sup>a</sup>	5,7 <sup>c</sup>	6,7 <sup>d</sup>	11,0 <sup>bc</sup>	6,1 <sup>c</sup>
Mediterranean	16	30,5	37,8 <sup>b</sup>	46,9 <sup>a</sup>	6,8 <sup>a</sup>	9,1 <sup>c</sup>	2,4 <sup>ab</sup>	3,7 <sup>bc</sup>	3,4 <sup>a</sup>	2,0 <sup>b</sup>	1,3 <sup>b</sup>	7,5 <sup>b</sup>	5,2 <sup>c</sup>	7,8 <sup>c</sup>	10,4 <sup>c</sup>	6,6 <sup>b</sup>
Middle East	18	28,7	38,3 <sup>b</sup>	35,3 <sup>b</sup>	5,2 <sup>b</sup>	6,7 <sup>a</sup>	2,7 <sup>a</sup>	3,3 <sup>cd</sup>	2,3 <sup>cd</sup>	2,2 <sup>b</sup>	1,5 <sup>b</sup>	7,8 <sup>b</sup>	6,4 <sup>b</sup>	10,4 <sup>a</sup>	13,6 <sup>a</sup>	7,4 <sup>a</sup>
F		1,2	6,0	18,5	13,7	12,1	10,0	12,2	5,5	5,2	4,7	8,0	10,1	17,2	3,9	9,5
p		>0.05	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.05	<0.001

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927 **Table 5.** Pearson correlation coefficient between 435 metabolic variables (*m/z*) and 15 phenotypic traits based on 12 ecotypes of *B. distachyon*,  
928 *B. stacei* and *B. hybridum*, \*, Bonferroni corrected significant p-values (< 0.05). \*\* many possible isomers with this particular accurate mass (see  
929 Table S9).

<i>m/z</i>	LGCL	PGL	H	NNTC	SLL	SLW	IL	NSS	SLA	SLB	NFS	UGL	LL	AL	CL	Putative Ionization Product	Adduct
n103.09	0.707	0.906 *	0.656	0.834	0.488	0.300	0.439	0.537	0.539	0.500	0.533	0.702	0.555	0.468	0.474	Hydroxy butyrate	[M-H]1-
n135	0.784	0.936 *	0.601	0.807	0.553	0.279	0.384	0.436	0.427	0.405	0.462	0.637	0.553	0.432	0.487	Threonate	[M-H]1-
n173.09	0.578	0.900 *	0.618	0.878	0.347	0.107	0.394	0.597	0.520	0.481	0.581	0.641	0.505	0.506	0.450	Shikimate	[M-H]1-
n174.09	0.724	0.929 *	0.617	0.885	0.429	0.101	0.345	0.533	0.444	0.405	0.488	0.633	0.497	0.437	0.519	<sup>13</sup> C Isotope of Shikimate	[M-H]1-
n191.09	0.605	0.919 *	0.610	0.856	0.429	0.189	0.423	0.608	0.530	0.492	0.588	0.682	0.534	0.496	0.463	Quinate	[M-H]1-
n192.09	0.590	0.897 *	0.646	0.856	0.394	0.218	0.460	0.628	0.587	0.552	0.633	0.707	0.547	0.528	0.474	<sup>13</sup> C Isotope of Quinate	[M-H]1-
n222.09	0.528	0.873	0.650	0.890 *	0.343	0.076	0.460	0.699	0.608	0.556	0.640	0.655	0.551	0.561	0.534	<i>N</i> -Acetyl tyrosine	[M-H]1-
n223.09	0.762	0.911 *	0.638	0.819	0.583	0.316	0.481	0.596	0.463	0.447	0.548	0.672	0.546	0.430	0.571	Sinapate	[M-H]1-
n236.09	0.658	0.714	0.823	0.571	0.573	0.421	0.752	0.614	0.835	0.808	0.730	0.643	0.891 *	0.768	0.690	<i>O</i> -Phospho homoserine	[M+K-2H]1-
n289.18	0.697	0.890 *	0.636	0.817	0.398	0.229	0.347	0.425	0.526	0.483	0.466	0.694	0.547	0.490	0.445	Sedoheptulose 7-phosphate	[M-H]1-
n420.18	0.472	0.632	0.811	0.571	0.539	0.661	0.866	0.780	0.874	0.876	0.891 *	0.760	0.756	0.700	0.630	β-1,4-Mannose- <i>N</i> -acetyl glucosamine	[M+K-2H]1-
n588.09	0.736	0.892 *	0.658	0.803	0.663	0.500	0.628	0.572	0.554	0.546	0.583	0.631	0.648	0.515	0.578	ADP-Glucose	[M-H]1-
n819.63	0.598	0.907 *	0.697	0.811	0.532	0.309	0.576	0.588	0.659	0.626	0.673	0.651	0.723	0.666	0.553	PG(18:1(11Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))**	[M-H]1-
n89.09	0.666	0.869	0.521	0.892 *	0.318	0.030	0.237	0.478	0.311	0.284	0.426	0.558	0.311	0.290	0.416	Oxalate	[M-H]1-
p445.27	-0.576	-0.751	-0.607	-0.534	-0.320	-0.352	-0.480	-0.519	-0.778	-0.764	-0.601	-0.904 *	-0.749	-0.723	-0.580	Unknown	-
p796.63	0.413	0.582	0.687	0.409	0.625	0.537	0.834	0.581	0.806	0.788	0.735	0.470	0.897 *	0.768	0.536	PC(16:0/18:2(2Z,4Z))**	[M+K]1+
p815.54	0.555	0.724	0.758	0.530	0.730	0.594	0.787	0.620	0.804	0.770	0.716	0.663	0.910 *	0.746	0.633	SQDG(16:0/16:1(11Z))**	[M+Na]1+
p816.54	0.548	0.747	0.708	0.493	0.699	0.593	0.790	0.664	0.820	0.803	0.765	0.724	0.913 *	0.758	0.607	PC(18:3(8E,10E,12E)/18:3(8E,10E,12E))**	[M+K]1+
p822.54	0.593	0.688	0.741	0.493	0.647	0.531	0.801	0.589	0.813	0.798	0.737	0.590	0.900 *	0.746	0.608	PC(18:0/18:3(9Z,12Z,15Z)/0:0)**	[M+K]1+
p823.54	0.690	0.692	0.840	0.585	0.668	0.478	0.772	0.563	0.809	0.776	0.682	0.591	0.904 *	0.745	0.764	PG(18:0/20:3(5Z,8Z,11Z))**	[M+Na]1+

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## LEGENDS TO FIGURES

**Figure 1.-** Geographic distribution of *B. distachyon* (blue), *B. stacei* (red) and *B. hybridum* (purple) samples used in the phenotypic and metabolomic study. Wild populations (circle), inbred lines (square), metabolomic samples (triangle).

**Figure 2.-** Box and whisker plots (median, percentiles, range) of 15 morphoanatomical characters analyzed in 1050 wild (grey) and inbred (white) individuals of the three species of *B. distachyon* s. l. complex. Variables that significantly discriminate *B. distachyon* vs. *B. stacei* vs. *B. hybridum* (A); *B. distachyon* vs. *B. stacei* + *B. hybridum* (B); *B. hybridum* vs. *B. distachyon* + *B. stacei* (C); variables that do not discriminate between species (D).

**Figure 3.-** Two-dimensional PCA plot of 46 wild populations of *B. distachyon* (blue), 31 of *B. stacei* (red) and 103 of *B. hybridum* (purple) based on averaged values of individuals analyzed for 15 phenotypic traits (see Tables 1, S2). The first and second PCA axes explain 79.2 % and 10.4 % of the total variation, respectively.

**Figure 4.-** Two-dimensional DA scatterplots of populations of *B. distachyon* (blue), *B. stacei* (red) and *B. hybridum* (purple) based on averaged values of individuals analyzed for 15 phenotypic traits in 174 wild populations (A) and in 174 wild populations plus 6 inbred lines (B). Wild populations (circles), inbred lines (stars), accessions with metabolites (*B. distachyon* diamonds; *B. stacei* triangle; *B. hybridum* squares).

**Figure 5.-** Two-dimensional DA scatterplots of 41 Spanish populations of *B. distachyon* (A), 29 circum-Mediterranean populations of *B. stacei* (B) and 89 circum-Mediterranean

populations of *B. hybridum* (C) based on averaged values of wild individuals analyzed for 15 phenotypic traits. Colors for the respective geographic groups of each species are indicated in the charts.

**Figure 6.-** Box- and whisker plots (median, percentiles, range) of a subsample of 16 significantly discriminant metabolomic traits analyzed in 5 ecotypes (60 replicates) of *B. distachyon* (blue), 3 (36) of *B. stacei* (red), and 4 (48) of *B. hybridum* (purple). (A) Positive mode metabolites; (B) Negative mode metabolites.

Assignment of metabolomics signals to putative ionization products is indicated for each trait.

**Figure 7.-** Two-dimensional DA scatterplot of 5 ecotypes (60 replicates) of *B. distachyon* (circles), 3 (36) of *B. stacei* (triangles) and 4 (48) of *B. hybridum* (squares) based on averaged values of individuals analyzed for 434 metabolomic traits. Colors for each ecotype are indicated in the charts.

## SUPPLEMENTAL MATERIAL

**Table S1.** Sources of *B. distachyon*, *B. stacei* and *B. hybridum* individuals from wild populations and inbred lines (\*) included in the phenotypic and metabolomic study. Double asterisks (\*\*) indicate individuals used in both types of analyses, in the remaining cases the individuals were used only in the phenotypic analysis. Diamonds indicate *B. distachyon*, *B. stacei* and *B. hybridum* type samples. Measurements of phenotypic variables correspond to averaged values from up to 10 individuals per population (and up to 5 measurements per trait and individual). The geographic origins and nature of all the studied samples are indicated [wild (W), herbaria (H), seed bank (S) or inbred (I) plants]. For abbreviation of variables see text and Table S2.

**Table S2.** Morphological variables used in the phenotypic analysis. Results of the Kolmogorov-Smirnov normality test. Significance: \*\*\*:  $p < 0.001$ ; \*\*:  $p < 0.01$ ; \*:  $p < 0.05$ ; n. s.: non-significant.

**Table S3.** Pairwise Spearman correlation coefficients between phenotypic traits (averaged values) analyzed in wild individuals of *B. distachyon*, *B. stacei* and *B. hybridum*. Significant correlation values ( $p < 0.001$ ) are highlighted in bold.

**Table S4.** Loadings of phenotypic traits onto the first PCA components. Highest contributions of characters to each component are highlighted in bold.

**Table S5.** Contribution of phenotypic variables to the first canonical discriminant functions obtained for the two data sets of individuals of *B. distachyon*, *B. stacei* and *B. hybridum*

analyzed: W (174 wild populations data set). I + W (6 inbred lines + 174 wild populations data set). Variables that contributed more to the functions are highlighted in bold.

**Table S6.** Contribution of phenotypic variables to the first canonical discriminant functions obtained for individuals from 41 wild populations of *B. distachyon*. Variables that contribute more to the functions are highlighted in bold.

**Table S7.** Contribution of phenotypic variables to the first canonical discriminant functions obtained for individuals from 29 wild populations of *B. stacei*. Variables that contribute more to the functions are highlighted in bold.

**Table S8.** Contribution of phenotypic variables to the first canonical discriminant functions obtained for individuals from 89 wild populations of *B. hybridum*. Variables that contribute more to the functions are highlighted in bold.

**Table S9.** Annotation of 434 ‘explanatory’ nominal mass fingerprint signals by targeted accurate  $m/z$  data analysis and MZedDB database search at MSI level of identification 2. The  $m/z$  signal with duplicate annotations, i.e., other adducts or isotopes of same metabolite, are indicated as plain (un-bold) text.

**Table S10.** List of annotated  $m/z$  signals where signal identities were confirmed by comparing  $MS^n$  fragmentation pattern with that of standard's.

**Table S11.** Contribution of metabolomic variables to the first canonical discriminant functions obtained for the studied ecotypes of *B. distachyon* (5 ecotypes, 60 replicates), *B.*

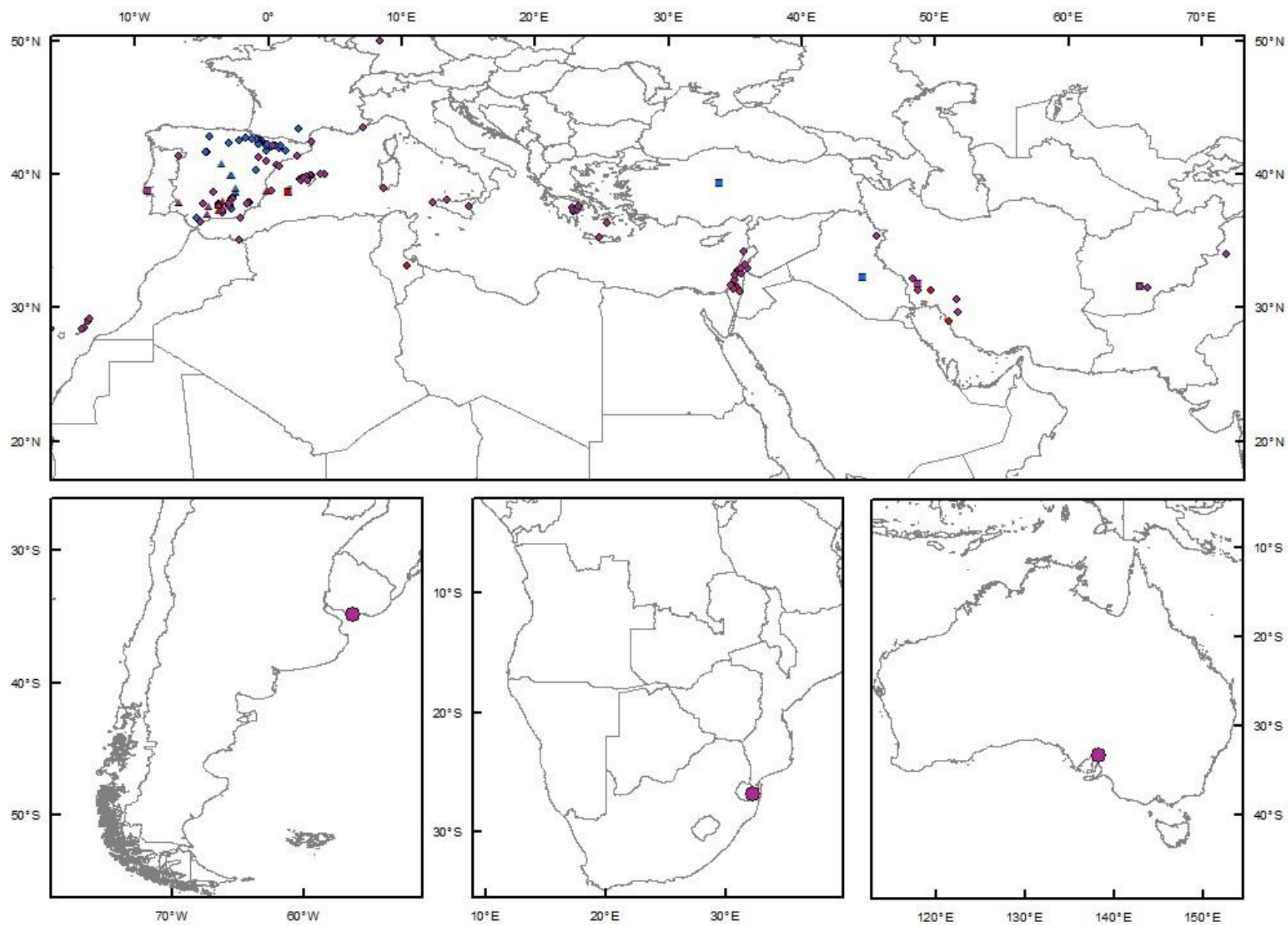
*stacei* (3, 36) and *B. hybridum* (4, 48). Contribution values of positive and negative metabolites to the functions. (a) Metabolites not included in the discriminant analysis (see Figure 7). (b) Duplicate *m/z* signals with same metabolite annotations not included in the discriminant analysis (see Figure S1). \*  $p < 0.05$ .

**Table S12.** Statistics of 16 metabolites and significance tests of their mean values in *Brachypodium distachyon*, *B. stacei* and *B. hybridum*. N, number of ecotypic samples analysed. ANOVA (F; d.f. 2) test of variables used for comparisons among species. Superscripts denote Tukey pairwise comparisons between species; means with the same letter do not differ significantly ( $p < 0.05$ ).

**Figure S1.** Two-dimensional DA scatterplot of 5 ecotypes (60 replicates) of *B. distachyon* (circles), 3 (36) of *B. stacei* (triangles) and 4 (48) of *B. hybridum* (squares) based on averaged values of individuals analyzed for 282 metabolomic traits (when *m/z* signals of same annotations showing different adducts were discarded from analysis; see Tables S9 and S11). Colors for each ecotype are indicated in the charts.

**Figure S2.** Tentative identification of main lipid in positive ion mode mass bin *m/z* 815.54 by MS<sup>2</sup> fragmentation analysis.

Fig. 1



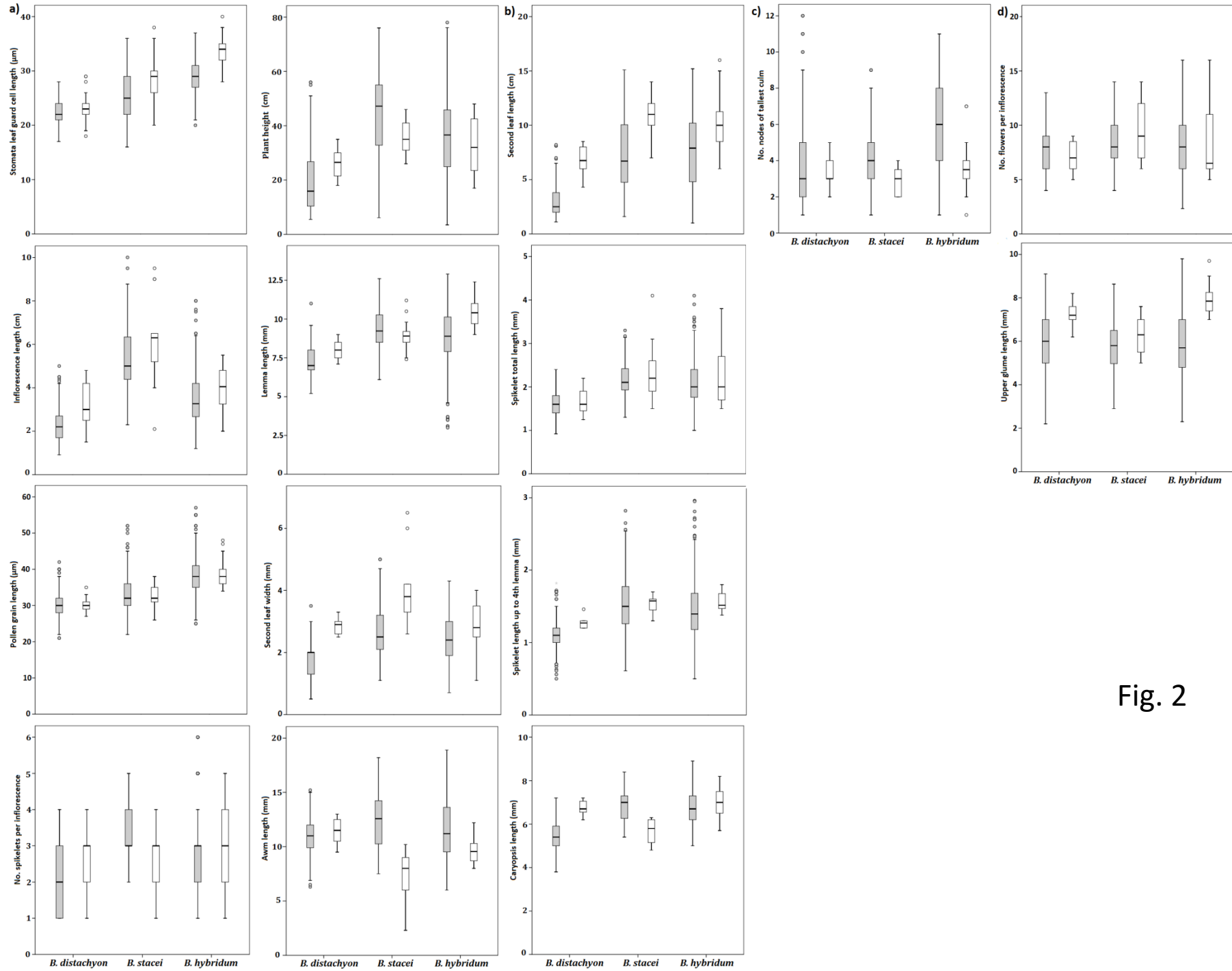


Fig. 2

Fig. 3

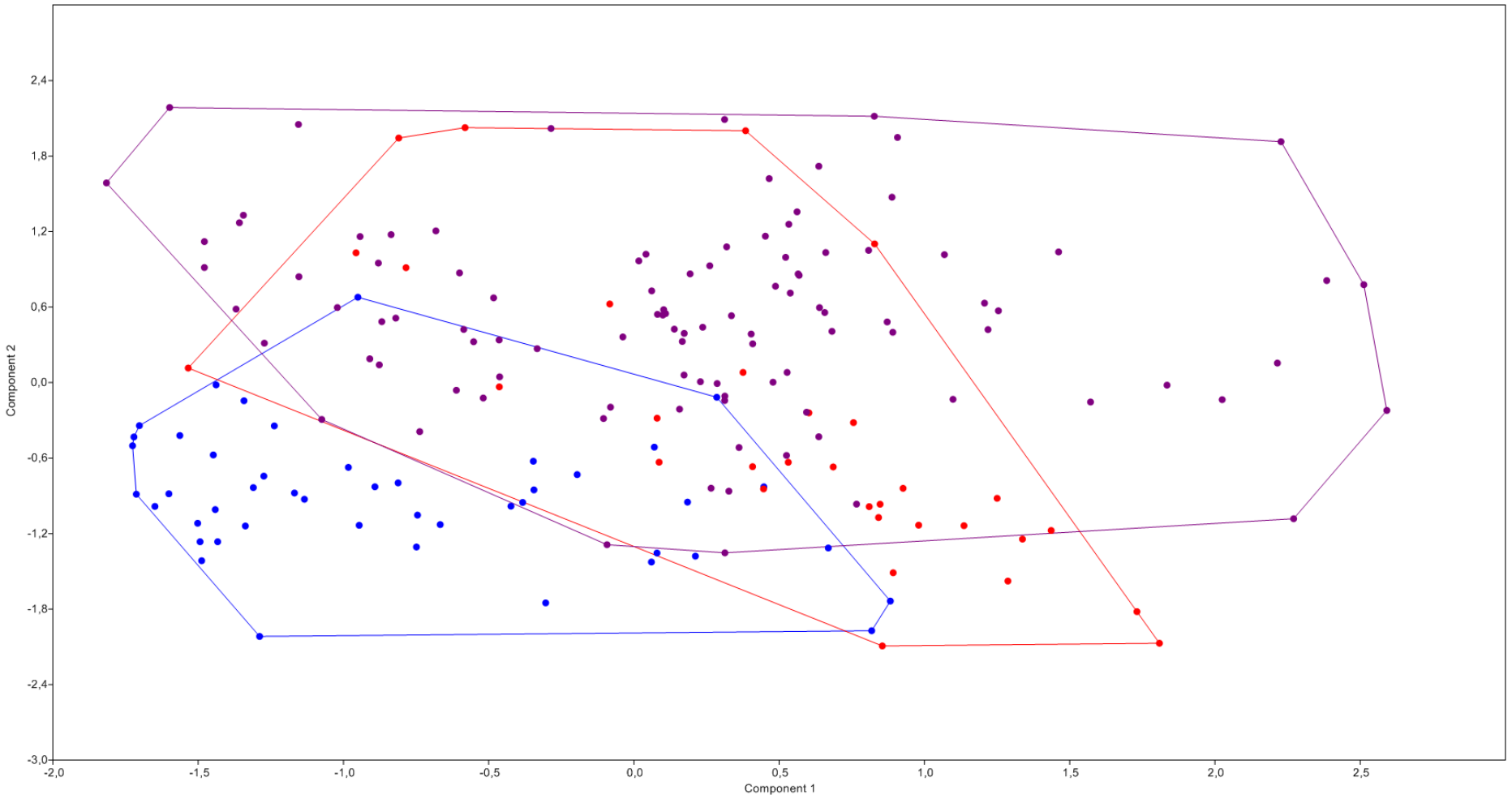
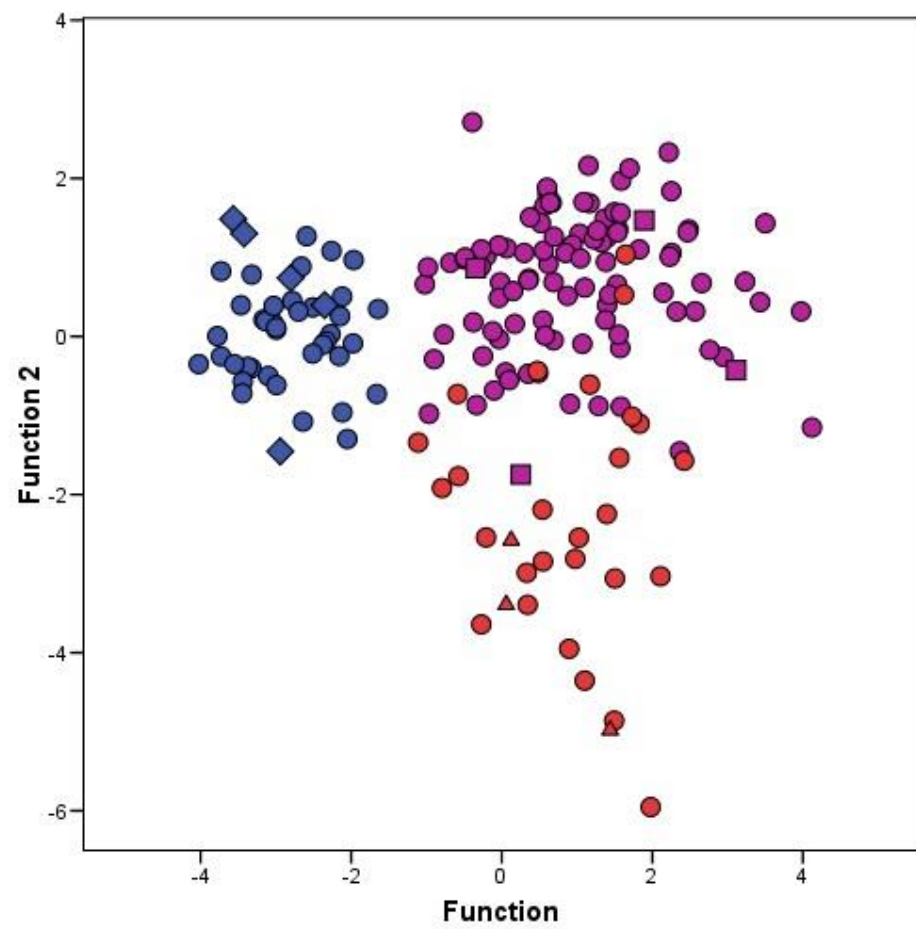




Fig. 4

A)



B)

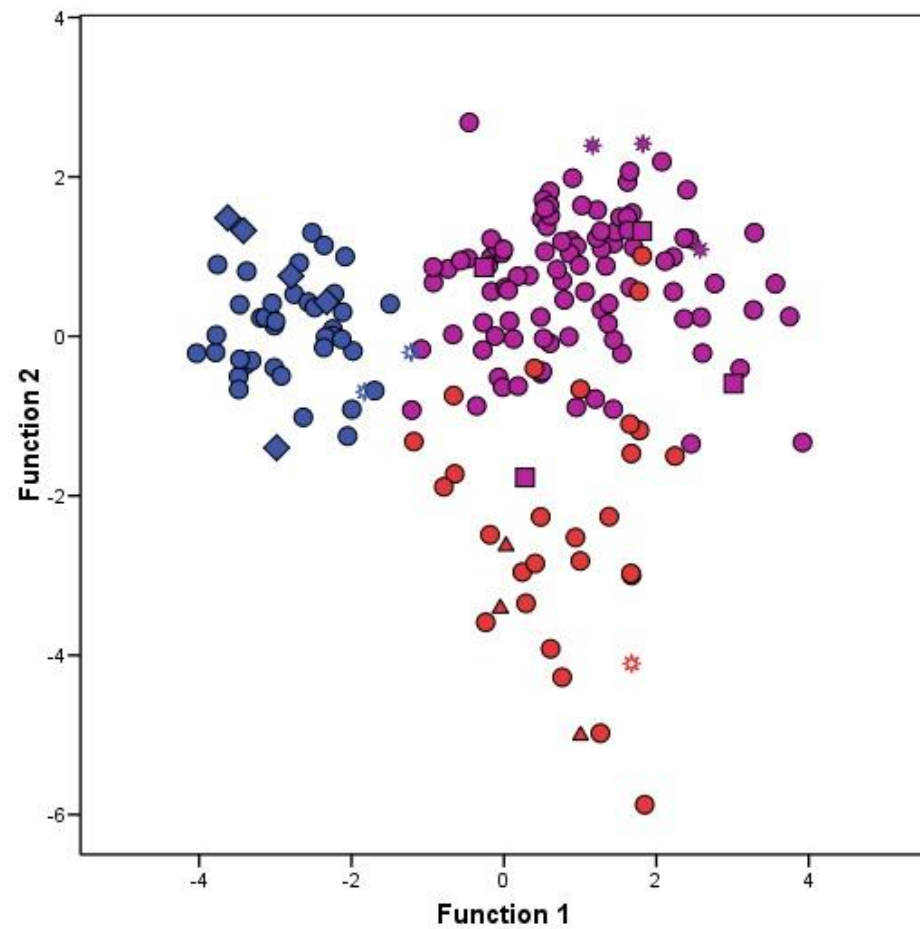
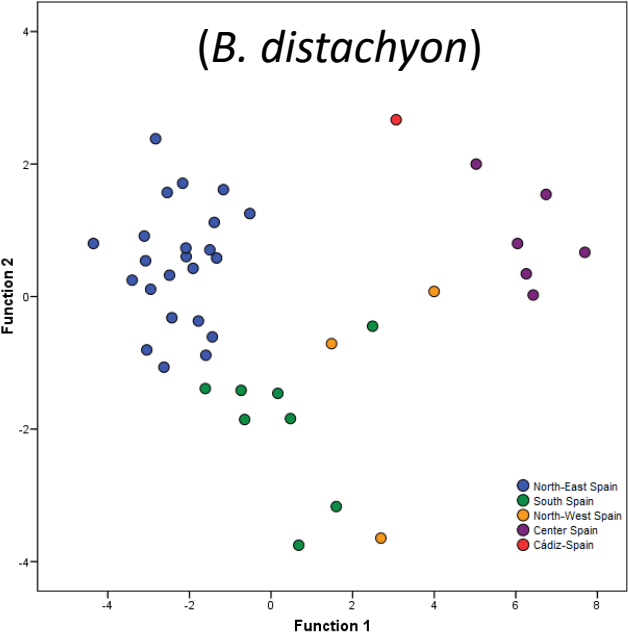
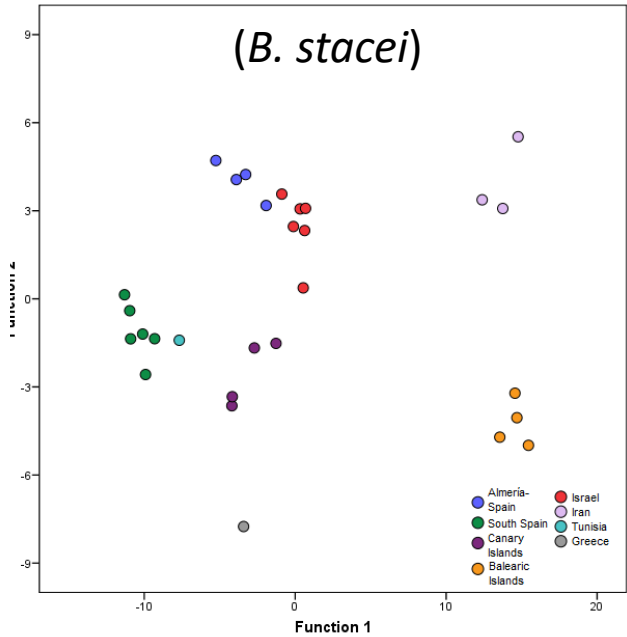


Fig. 5

A)



B)



C)

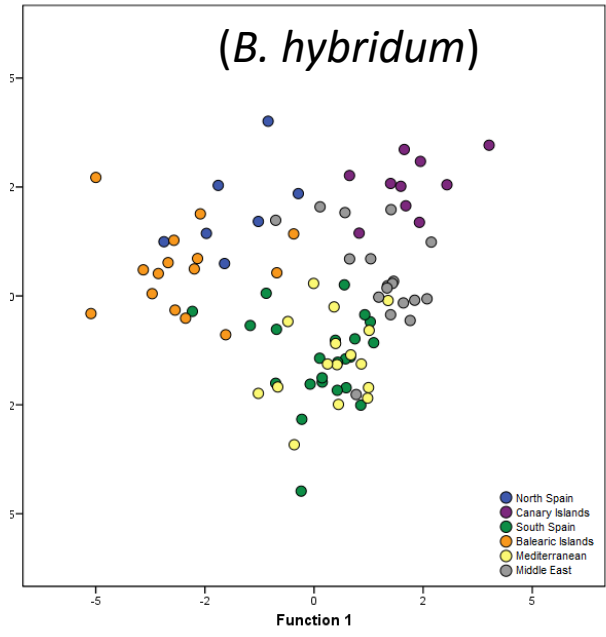


Fig. 6

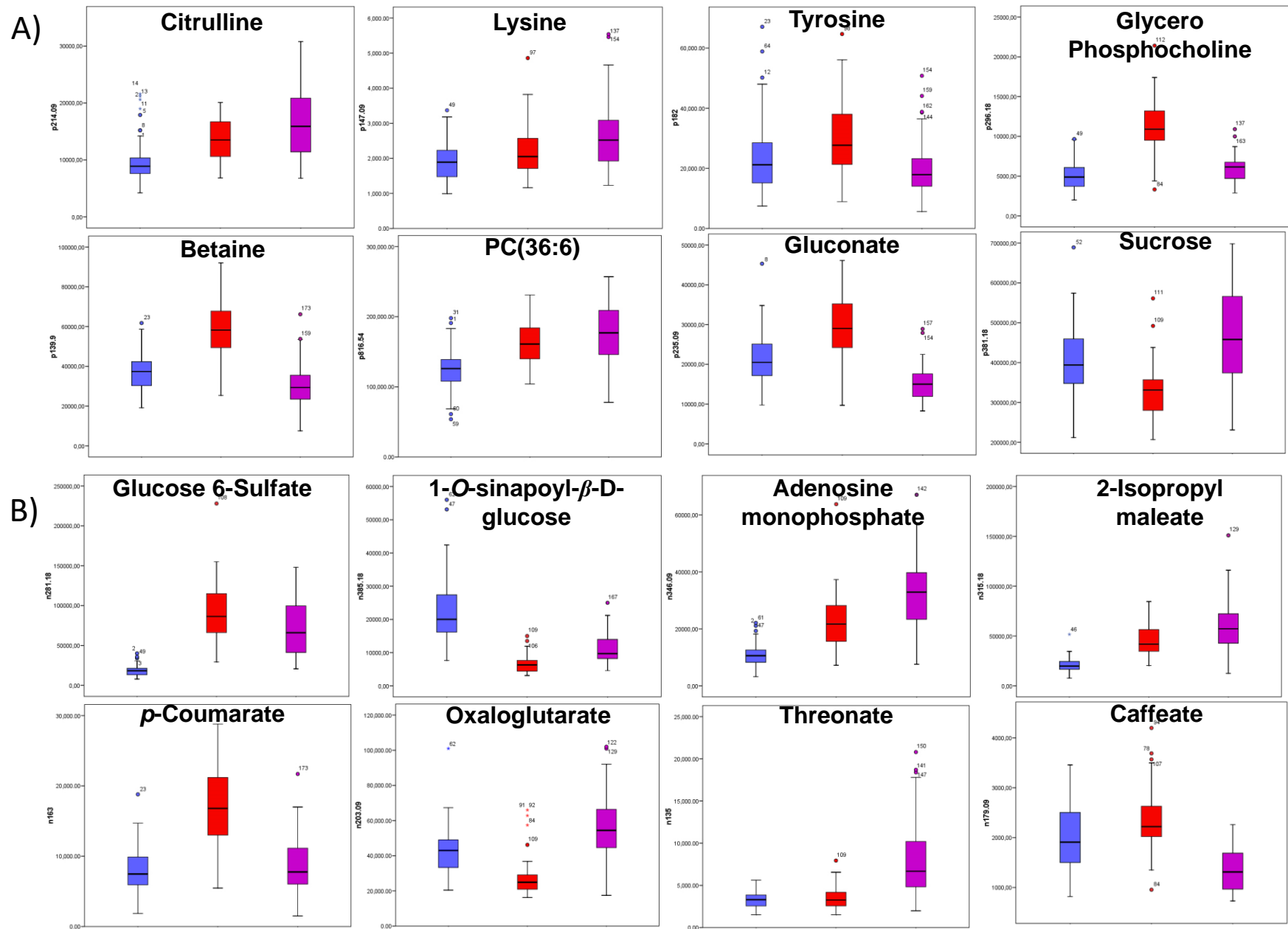
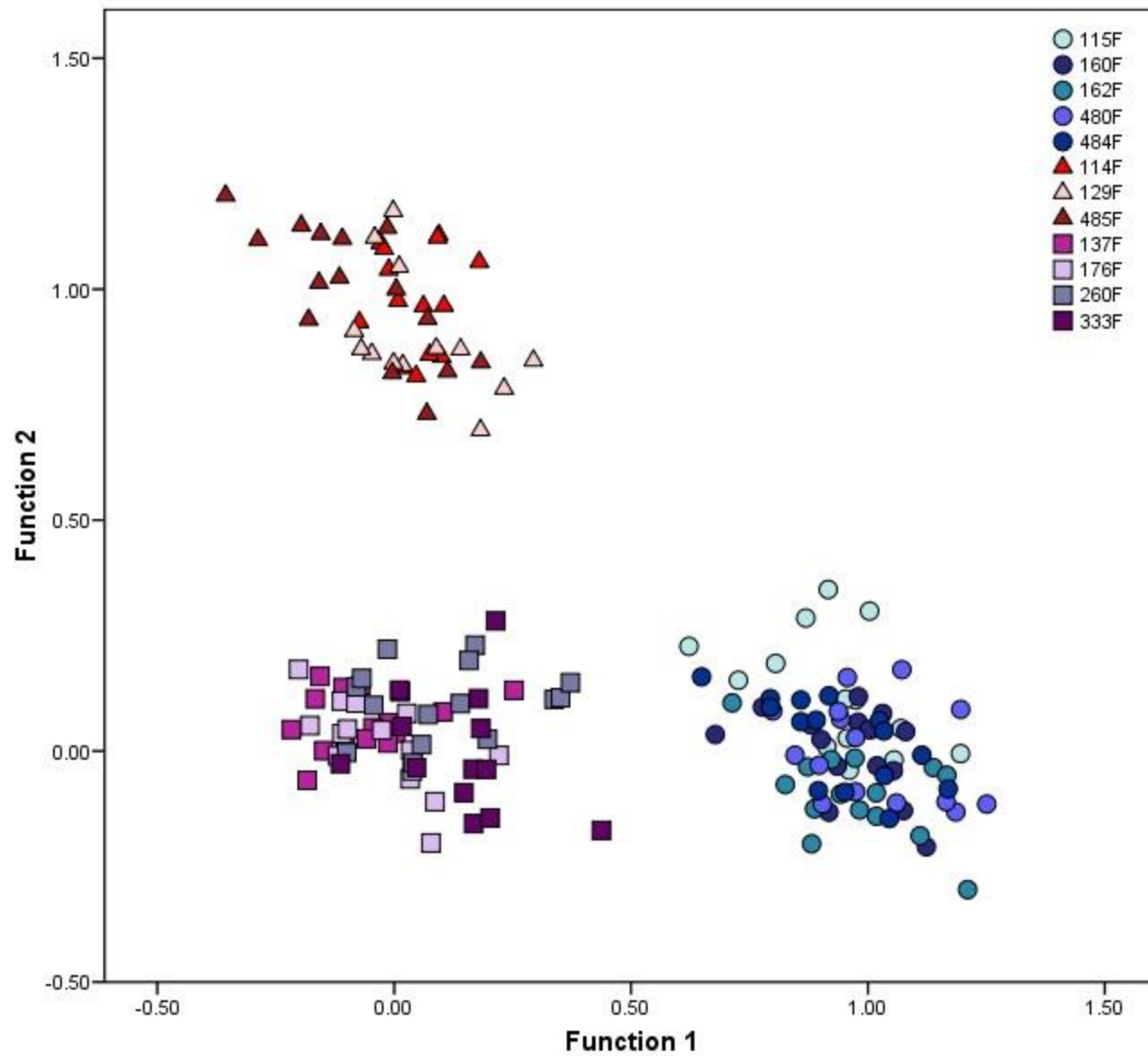


Fig. 7



**Table S1.** Sources of *B. distachyon*, *B. stacei* and *B. hybridum* individuals from wild populations and inbred lines (\*) included in the phenotypic and metabolomic study. Double asterisks (\*\*) indicate individuals used in both types of analyses, in the remaining cases the individuals were used only in the phenotypic analysis. Diamonds indicate *B. distachyon*, *B. stacei* and *B. hybridum* type samples. Measurements of phenotypic variables correspond to averaged values from up to 10 individuals per population (and up to 5 measurements per trait and individual). The geographic origins and nature of all the studied samples are indicated [wild (W), herbaria (H), seed bank (S) or inbred (I) plants]. For abbreviation of variables see text and Table S2.

Specie	Locality	LGCL	PGL	H	NNTC	SLL	SLW	IL	NSS	SLA	SLB	NFS	UGL	LL	AL	CL
<i>B. distachyon</i> *♦	Iraq: Bd21 USDA genome sequenced (type) (H)	22,3	30	28	3	6,3	2,9	3	2,5	1,7	1,3	7,1	7	8	12	7
<i>B. distachyon</i>	France: Aude. USDA:W619177 (S)	22,8	33	32	6,6	3,5	0,9	2	1,8	1,5	1	6,3	3,9	6,6	7,9	5
<i>B. distachyon</i>	Israel: Julis. AB&SE:BRA65 (S)	21,4	30	47	6	4,3	3,5	4	3	2,4	1,8	13	7	8,7	13	5
<i>B. distachyon</i>	Spain: Albacete, Alcaraz. CS:Bd115F** (I)	20,8	29	50	6,6	5,6	1,8	3	3,5	1,8	1,3	6,4	7,4	8,1	11	6
<i>B. distachyon</i>	Spain: Albacete, Bonillo. CS:Bd160F** (I)	21,4	30	39	5,7	4,8	2	3	3	1,7	1,4	8	8	7,9	11	6
<i>B. distachyon</i>	Spain: Cádiz, Grazalema. AM:Graz (S)	21,4	35	36	4	2,6	1,8	4	3	2	1,6	13	6,7	7,6	14	7
<i>B. distachyon</i>	Spain: Cuenca, Sedóbriga. CS:Bd162F** (I)	21,3	33	38	7	3,5	1,8	3	3	1,7	1,4	6,5	7,5	8,4	13	6
<i>B. distachyon</i>	Spain: Cuenca. CS:Bd484F** (I)	20	30	37	7,3	5,8	1,6	2	2,7	1,4	1	6	6,4	7,1	9,8	6
<i>B. distachyon</i>	Spain: Escaray, Zorraquín, monte Turgaiza.HS:100198-1 (S)	24,8	36	40	5,3	8,1	2,3	2	2,8	1,4	0,9	10	4,4	6,1	7,5	5
<i>B. distachyon</i>	Spain:Granada, Sierra Huetor, Puerto de la Mora. PC:B111 (W)	22	31	15	4	1,5	0,9	1	1	1,2	0,9	6	4,1	6,2	8,9	6
<i>B. distachyon</i>	Spain: Granada, Sierra de Baza, Mirador de Narvaez1. PC Bsta128 (W)	24,4	28	24	10,8	5,3	2,2	2	1,2	1,5	1	5,6	3,6	6,9	10	5
<i>B. distachyon</i>	Spain: Granada, Sierra de Baza, Mirador de Narvaez 2. PC:B129 (W)	21,3	28	14	2,7	1,9	1,6	3	2,7	1,8	1,4	9,7	4,9	7	8,4	6
<i>B. distachyon</i>	Spain: Granada, Sra Nevada, Ctra al Veleta, ca. Restaurante Las Viboras. PC:B136 (W)	26	27	9,3	3,7	1,6	1,1	2	1	1,6	1,2	7,3	3,4	7,4	12	6
<i>B. distachyon</i>	Spain: Huesca, Jaca, Banaguas. PC&LM:B12 (W)	19,2	30	24	3,2	3,3	2,3	2	3,3	1,5	1,2	6,6	5,8	6,3	11	5
<i>B. distachyon</i>	Spain: Huesca, Puente de la Reina. PC&LM:B14 (W)	21,4	26	11	2,4	2,8	2	3	2,6	1,9	1,2	10	7	8	11	5
<i>B. distachyon</i>	Spain: Huesca, Arens. PC&LM:B18 (W)	27	27	14	2	2,5	2	3	2,4	1,8	1	9,2	7,2	6,6	11	5
<i>B. distachyon</i>	Spain: Huesca, Adahuesca, San Jerónimo. PC&LM:B19 (W)	24	29	17	2	2,4	2	3	2,5	1,9	1,2	9,5	6,5	7,3	13	5
<i>B. distachyon</i>	Spain: Huesca, Barbastro. PC&LM:B20 (W)	21,8	32	30	4,2	4,2	2,3	3	2,6	1,9	1,1	8,8	7,6	8,5	13	5
<i>B. distachyon</i>	Spain: Huesca, Abizanda. PC&LM:B21 (W)	26,3	30	19	2,4	2,7	2	3	2	1,7	1,2	8	6,2	7,4	13	6
<i>B. distachyon</i>	Spain: Huesca, Berdun. PC&LM:B22(W)	25	29	16	2,7	2,8	2	2	2,6	1,5	1	7,7	6,2	6,8	11	5
<i>B. distachyon</i>	Spain: Huesca, Bierge. PC&LM:B09 (W)	21	32	12	3,2	1,8	1,4	2	1,4	1,4	0,9	6,6	4,9	7,2	11	5
<i>B. distachyon</i>	Spain: Huesca, Alquezar. PC&LM:B11 (W)	21,5	41	19	3,2	2,6	1,9	3	1,8	2,1	1,6	11	8,5	7,3	14	6

<i>B. distachyon</i>	Spain: Huesca, Benabarre. PC&LM:B17 (W)	22,5	35	13	3,5	2,9	1,8	2	2,8	1,4	0,8	6,8	7,2	7,1	11	6
<i>B. distachyon</i>	Spain: Huesca, Sariñena. PC&LM:B05 (W)	22,4	32	7,4	2	1,5	1,6	2	2,2	1,7	1,1	9,6	6,8	7,6	8,2	5
<i>B. distachyon</i>	Spain: Huesca, San Miguel de Foces. PC&LM:B06 (W)	21,8	35	11	3	1,8	1,8	2	2,2	1,8	1,3	10	6	6,6	10	5
<i>B. distachyon</i>	Spain: Huesca, Yaso. PC&LM:B08 (W)	22,4	33	15	4,5	2,8	1,7	3	2	1,6	1,3	8,5	6	6,8	11	7
<i>B. distachyon</i>	Spain: Jaen, Sierra Sepura, Cortijos Nuevos, El Contadero. PC B115 (W)	21,2	29	20	9,2	3,6	1,4	2	1,2	1,4	0,9	4,6	4,2	7	8,1	6
<i>B. distachyon</i>	Spain: Jaen, Sierra Sepura, Cortijos Nuevos, El Contadero. PC:B116 (W)	23,8	31	22	5,8	4	2	2	1,4	1,3	0,8	5	4,5	6,8	9,4	6
<i>B. distachyon</i>	Spain: Jaén, Sra de Jaen, zona de pitillos. PC:B134 (W)	24,4	32	30	4,2	4,8	1,8	2	2,2	1,5	1	6,4	4,6	7,4	9,1	6
<i>B. distachyon</i>	Spain: Jaén, Sra de Jaen, Puerto de Navalayegua. PC:B135 (W)	20,7	22	15	3,3	2,1	1,5	3	2,3	1,9	1,3	9,3	5,3	8,4	10	7
<i>B. distachyon</i>	Spain: Teruel, La Puebla de Valverde, Pto del Escandón. PC:B142 (W)	23,3	31	7	2,8	2	1,6	2	2,3	1,7	1,3	9,8	3,4	7,5	8,3	6
<i>B. distachyon</i>	Spain: Lleida, Fondedou . PC&LM:B30 (W)	21,5	29	7,5	2,3	2,1	1	2	2	1,4	1	7,3	6,5	6,8	11	4
<i>B. distachyon</i>	Spain: Lleida, Castillo de Mur. PC&LM:B31 (W)	24,6	28	23	2,8	4,3	2,7	3	3	1,8	1,1	9,6	7,4	7,8	13	5
<i>B. distachyon</i>	Spain: Lleida, Les Pallagues. PC&LM:B32 (W)	25	26	11	2	2,1	1,5	2	1,4	1,6	1,1	9	6,8	7,3	12	5
<i>B. distachyon</i>	Spain: Madrid. CS:Bd480F** (I)	20,4	27	50	7	5,1	2,1	4	3	1,4	1,1	7	6,3	7,1	11	6
<i>B. distachyon</i>	Spain: Navarra, Puerto del Perdon. PC&LM:B27 (W)	23,4	32	9,7	2,2	1,9	1,4	2	1,5	1,5	1	8,2	5,8	6,8	11	5
<i>B. distachyon</i>	Spain: Navarra, Los Arcos. PC&LM:B28 (W)	25,2	27	8,5	2,8	2	2	2	1,8	1,7	1,1	8,6	6,8	8,2	11	5
<i>B. distachyon</i>	Spain: Navarra, Puerto del Perdon. PC&LM:B27 (W)	23,4	32	9,7	2,2	1,9	1,4	2	1,5	1,5	1	8,2	5,8	6,8	11	5
<i>B. distachyon</i>	Spain : Otero, Valladolid. INRA-V:Abrc7d (S)	25	30	29	9,8	3,8	1,8	2	1	1,5	1	4,6	4,4	7,5	9,2	6
<i>B. distachyon</i>	Spain: Palencia, Cervera. AM:Cer (S)	19,8	30	37	6	2,3	1,8	4	3,3	2	1,4	12	7,3	7,1	11	5
<i>B. distachyon</i>	Spain: Valladolid,Renedo de Esgueva. HS:113171-1 (S)	21,6	26	31	6	6,9	2,7	2	3	1,4	0,7	7	6,5	6,4	9,4	4
<i>B. distachyon</i>	Spain: Zaragoza, Miramont. PC&LM:B23 (W)	24,2	26	11	3	1,5	1	2	1,1	1,4	1,1	5,8	6	6,7	11	5
<i>B. distachyon</i>	Spain: Zaragoza, Sigüés. PC&LM:B24 (W)	23,4	26	12	2,6	1,9	1,8	2	2,1	1,7	0,9	8,6	6,4	7,1	10	6
<i>B. distachyon</i>	Spain: Zaragoza, Murillo de Gallego. PC&LM:B15 (W)	24,6	30	6,9	1,3	2,3	2	2	2,2	1,8	1	9	7,3	8,2	11	5
<i>B. distachyon</i>	Turkey: Kiresehir, Kaman. ABR1* (I)	24,6	29	20	4,5	8,3	2,8	4	3,3	1,6	1,3	6,7	8	8,3	9,9	7
<i>B. distachyon</i>	Spain (S)	19,2	35	43	4,7	2,5	1,4	4	2,7	2	1,3	8,7	6,9	8,9	12	6
Specie	Locality	LGCL	PGL	H	NNTC	SLL	SLW	IL	NSS	SLA	SLB	NFS	UGL	LL	AL	CL
<i>B. stacei</i> *∅	Spain: Balearic isles: Formentera. ABR114* (type) (I)	28,2	33	36	2,9	11	4,2	6	2,8	2,3	1,6	9,2	6,2	8,9	7,3	6
<i>B. stacei</i>	Greece: Pelopponesos, Tyros. PC:G15 (W)	19,7	29	65	5,8	11	2,8	6	4,3	1,8	0,9	7,8	5,2	10	14	6
<i>B. stacei</i>	Iran: Asalooyeh to Booshehr MRR:BdisIran1 (H)	30	45	20	3,3	3,5	1,8	4	2	2,7	2,4	13	5,1	9,3	13	8

<i>B. stacei</i>	Iran: 25 Km to Ramhorm from Ahwaz. MRR:BdisIran2 (H)	28,3	47	24	3	4,4	3,3	5	3	2,5	1,9	11	6	8,4	14	8
<i>B. stacei</i>	Iran: Fars, Kazeroon, Davan. MRR:B100-H141 (H)	27,3	49	39	4,7	5,7	1,8	3	2	2,7	2	11	5,6	10	17	8
<i>B. stacei</i>	Israel: Amazyia. AB&SE:BRA52 (S)	23,8	31	49	5	6	2,4	6	4	2,6	1,8	9	6,3	12	15	7
<i>B. stacei</i>	Israel: Lakhish. AB&SE:BRA135 (S)	32,7	28	36	4	6,1	2,8	7	4	1,3	1,2	8	6,1	9,9	14	6
<i>B. stacei</i>	Israel: Hawalid. AB&SE:BRA158 (S)	21,2	34	42	7	3,4	2,3	5	3	2,1	1,7	7	7,2	11	13	7
<i>B. stacei</i>	Israel: Bat Shelomo. AB&SE:BRA191 (S)	27	40	33	6	3,3	2	5	3	1,9	1,1	6	5,8	8,6	15	7
<i>B. stacei</i>	Israel: Yiftah. AB&SE:BRA286 (S)	28,4	43	47	9	3,6	3	5	3	3,3	2,5	14	7,1	9	16	7
<i>B. stacei</i>	Israel: Ziff junction. AB&SE:BRA89 (S)	21	38	47	4	7,9	2,7	5	3	2,1	1,7	8	5,7	10	18	7
<i>B. stacei</i>	Spain: Alicante, Xávea, Cabo La Nao. PC:B102 (W)	17,1	32	56	5,3	12	3,1	7	3,9	2	1,1	5,6	6,1	8,9	12	7
<i>B. stacei</i>	Spain: Alicante, Cabo de La Nao. CS:Bd483F** (I)	23,3	31	57	6,3	9,9	3,9	7	3,7	2,3	1,9	9,3	8,3	9,7	13	6
<i>B. stacei</i>	Spain: Almería, Cabo de Gata. EB:AL1 (S)	24	31	54	4,5	6,4	3,4	7	4	2,8	2,2	12	4,7	10	16	8
<i>B. stacei</i>	Spain: Almería, Cabo de Gata. EB:AL5 (S)	21,5	38	45	4	5,1	2,3	5	2,5	2,9	2,4	12	5	9,1	13	7
<i>B. stacei</i>	Spain: Almeria, Cabo de Gata, Playa de Genoveses. PC B108 (W)	30,5	30	44	2,8	5,9	2,8	5	3,3	2,5	1,6	8,4	5,2	11	9,3	6
<i>B. stacei</i>	Spain: Almeria, Cabo de Gata, Playa de Monsal. PC B109 (W)	31,6	34	41	3,2	8,4	2,6	5	2,8	2,4	1,5	8,1	5,2	10	10	6
<i>B. stacei</i>	Spain: Canarias: Gomera, Agulo. INIA-CRF: NC050363 (W)	26,5	33	46	4	7,8	2,7	5	4	1,9	1,2	7,3	5,3	9,4	14	7
<i>B. stacei</i>	Spain: Canarias: Gomera, Agulo. INIA-CRF:BGE044241 (W)	21,8	30	50	4,5	5,1	2,4	5	2,5	2,4	1,6	8	7,8	10	11	6
<i>B. stacei</i>	Spain: Canarias: Lanzarote, Teguiuse. INIA-CRF:NC050440 (S)	25	31	49	4,7	7,6	2,2	6	4	2,2	1,6	10	4,3	7,9	9,1	7
<i>B. stacei</i>	Spain: Canarias: Lanzarote, Teguiuse. INIA-CRF:BGE044249 (S)	26,8	32	51	3	4	2,5	6	3,5	2,1	1,4	9	5,8	9,1	8,7	7
<i>B. stacei</i>	Spain: Granada, Moclin. CS:Bd129F**(I)	20,2	30	64	6,8	7,6	2,2	6	3,4	2,4	1,9	9,4	7,2	11	16	8
<i>B. stacei</i>	Spain:Jaen, Sierra Cazorla, Ctra de Cortijos Nuevos a Cazorla. PC:B117 (W)	22	25	49	5	11	3,2	6	3,6	2	1,3	5,2	5,2	9,8	14	7
<i>B. stacei</i>	Spain: Jaen, Baeza. CS:Bd114F** (I)	24,9	31	51	6,7	7,4	2,2	6	3,3	1,9	1,5	7,7	6,5	8,9	12	6
<i>B. stacei</i>	Spain: Jaen, Sierra de Quesada. Tiscar. JD:18_1 (S)	26,5	31	59	7,5	7,3	2,6	5	3,5	1,8	0,9	6	4,9	9	18	7
<i>B. stacei</i>	Spain: Mallorca, Sa Dragonera, Gambes. DL&PC:B891 (W)	27,9	33	9,7	1,8	2,8	1,5	4	3	1,9	1,3	9	4,8	7,6	8,9	6
<i>B. stacei</i>	Spain:Mallorca, Bahyalbufear, Mirador Ses Animes. DL&PC:B921 (W)	29	39	21	4	4,7	2,2	4	2,5	2,2	1,7	11	6,7	8,6	14	7
<i>B. stacei</i>	Spain: Mallorca, Alcudia, c. Punta Negra. DL&PC:B621 (W)	28,6	40	18	2,7	4,8	2,7	3	2,3	2	1,3	8,7	5,4	8,2	10	6
<i>B. stacei</i>	Spain: Mallorca, Campanet, Coves. DL&PC:B771 (W)	24,8	35	27	2,8	5,2	3	4	2,3	2,2	1,6	10	6,2	10	13	6
<i>B. stacei</i>	Spain: Mallorca, La Victoria. INRA:13498BGVA_Esp0 (W)	27,6	31	55	3,4	12	4,1	5	3,2	1,9	1,3	6	5,3	9,1	9,6	8

<i>B. stacei</i>	Tunisia: Gouv. De Medénine:Ksar Hadada (NW Lfoum Tataouine). Hmünchen:67243 (H)	28,6	31	42	4	4,2	1,2	5	3	2,2	1,7	10	5,7	7,4	12	6
Specie	Locality	LGCL	PGL	H	NNTC	SLL	SLW	IL	NSS	SLA	SLB	NFS	UGL	LL	AL	CL
<i>B. hybridum</i> *♦	Portugal: Lisboa, ABR113. (type)* (I)	34	36	48	7	13	2,6	4	4,5	1,5	1,5	6	8,8	10	12	7
<i>B. hybridum</i>	Afghanistan: Hauz-i-Mabat, Kandahar. USDA:PI219965 (S)	29,6	37	38	4,2	8	2,3	3	2,4	1,9	1,2	7,6	5,4	11	14	8
<i>B. hybridum</i>	Afghanistan: 35 miles from Kandahar to Kahakai dam, 4,800 ft. USDA:PL219971 (S)	33,5	36	35	5,3	9,8	3,2	3	2,3	2	1,4	6,5	5,5	11	13	8
<i>B. hybridum</i>	Afghanistan: USDA PI219965, ABR117* (I)	34,1	38	22	4	7,5	1,5	3	1,4	2,3	1,6	8,8	7,7	10	9,2	7
<i>B. hybridum</i>	Australia: Crystal Brook, 3 km upstream from town. USDA:PL533015 (S)	32,6	38	35	8	10	3,2	3	2,4	1,9	1,3	6,6	4,6	8,4	9,1	7
<i>B. hybridum</i>	Crete. INRA:Cre0 (S)	32,6	36	78	4	4,4	1,7	5	3,3	3,4	2,5	13	6,8	8,6	9,9	9
<i>B. hybridum</i>	Crete. INRA-V:Cre-1a(S)	36	36	48	7,2	12	3	3	3,8	1,7	1,1	5,5	5,8	9,6	9,2	7
<i>B. hybridum</i>	Crete. INRA-V:Cre-1b (S)	33,2	42	42	7,4	11	3,3	3	3,5	1,6	1	5,5	4,9	8,8	8,4	6
<i>B. hybridum</i>	Cerdeña: Cagliari. HMünchen:55806 (H)	27,1	39	68	5	7,5	1,8	5	4,5	1,9	1,4	9,5	5,2	6,9	11	6
<i>B. hybridum</i>	France: Île sainte Marguerite. INRA-V:Mar-1 (S)	30	37	36	9,4	11	2,9	3	2,8	1,6	1,1	5,2	5,1	8,4	11	7
<i>B. hybridum</i>	Germany. USDA:PL422452 (S)	29,8	42	42	8,6	9,3	3,1	3	2	1,8	1,3	6,2	5,3	8,9	8	6
<i>B. hybridum</i>	Grece: Kreta, Metohi. HMünchen:201179 (H)	30,7	40	54	5	7,6	2,1	5	3,5	1,8	1,4	8,3	6	7,8	12	8
<i>B. hybridum</i>	Greece: Pelopponesos: Xiropigado.PC:G12 (W)	30,4	35	53	7,5	8,3	2,4	5	5,5	1,6	0,8	6	5,6	6,7	10	7
<i>B. hybridum</i>	Greece: Pelopponesos: Korakovouni, Aghios Andreas.PC:G13 (W)	27,4	40	54	5,5	9,7	2,4	5	5,5	2,7	1,5	12	4,9	7,4	11	7
<i>B. hybridum</i>	Greece: Pelopponesos: Tyros.PC:G14 (W)	34,1	38	55	7	9,5	2,6	5	5,5	1,9	0,9	7,5	4,1	6,9	11	6
<i>B. hybridum</i>	Greece: Pelopponesos: Kosta1. PC:G3 (W)	29,3	39	45	7	12	2,4	3	3,7	1,4	0,9	6	4,5	5,9	10	6
<i>B. hybridum</i>	Greece: Pelopponesos: Ano Fanari.PC:G8 (W)	27,3	39	41	7	5,5	1,7	4	2	2,2	1,7	11	5,9	8,6	10	6
<i>B. hybridum</i>	Iran: Fars, Kazerun , 12 miles E of Kazerum, 3,800 ft. USDA:PL226629 (S)	33,5	41	47	4,3	8,9	2,8	4	3	2,4	1,8	9,5	5,6	11	15	7
<i>B. hybridum</i>	Iran: Juzestan, between Ahwaz and Shushtar, near Abe-e Gonji. USDA:PL227011 (S)	28,4	35	39	4,4	9,2	3,1	4	3,2	2	1,4	5,5	6,2	11	14	7
<i>B. hybridum</i>	Iran: Susa, SW Iran. USDA:PL239715 (S)	32	41	39	5	8,1	1,6	3	2	1,9	1,5	5	3,9	10	13	8
<i>B. hybridum</i>	Iran: Kohkiloyeh, Gchaemich T.Chogan. MRR:B99-H138 (H)	27,2	36	27	4,7	4,6	1,8	4	2,7	2,1	1,6	12	7,6	9	17	6
<i>B. hybridum</i>	Iran: Kohkiloyeh, Yasooj. MRR:B98-H137 (H)	30,8	51	70	6	6,2	2,8	3	1	3,3	2,7	14	7,2	9,7	18	8
<i>B. hybridum</i>	Iran: Khūzestān, Kalafabad. ABR100* (I)	33,2	41	41	3,1	11	3,3	4	3,2	2,3	1,6	8	8	11	9,6	8
<i>B. hybridum</i>	Iraq: As Sulaymāniyah, Arbet, 30 km S of Arbet. USDA:PL254868 (S)	29,4	37	37	6,4	8,4	3,1	2	1,2	1,8	1,3	5,8	6,6	11	15	7



<i>B. hybridum</i>	Israel: Lehavim. AB&SE:BRA7 (S)	31	38	36	7	4,2	3	4	3	2,3	1,7	8	7,3	9,6	14	9
<i>B. hybridum</i>	Israel: Gilbo'a. AB&SE:BRA26 (S)	26,3	39	35	5	10	3,1	3	1	3,1	1,8	8	5	13	14	7
<i>B. hybridum</i>	Israel: Nahal Bokek. AB&SE:BRA57 (S)	28	34	27	3	3,5	3,1	3	1	2,6	1,9	8	8,2	10	13	8
<i>B. hybridum</i>	Israel: Petah Tikwa. AB&SE:BRA143 (S)	28,7	33	37	5	6,5	2,4	4	3	2,3	1,2	9	7,7	12	13	7
<i>B. hybridum</i>	Israel: Amirim. AB&SE:BRA146 (S)	22,3	40	20	4	8,5	2,8	3	2	2,1	1,3	7	7,6	8,5	13	8
<i>B. hybridum</i>	Israel: Tel Fares. AB&SE:BRA160 (S)	24,6	36	33	5	2,9	1,9	3	3	1,8	1,1	6	8,8	12	16	8
<i>B. hybridum</i>	Israel: Brachyia. AB&SE:BRA221 (S)	27	49	29	6	3	2,4	3	2	2,2	1,6	9	4	11	12	7
<i>B. hybridum</i>	Israel: Sion. AB&SE:BRA293(S)	23,2	44	43	8	3,1	2,2	5	3	2,4	1,8	11	7,5	10	16	7
<i>B. hybridum</i>	Israel: Tel Aviv Univ, Botanical Garden. AB&SE:BRA299 (S)	25,8	34	27	5	2,5	3,9	3	2	2,1	1,4	9	7,2	9,3	7,3	7
<i>B. hybridum</i>	Italy: Sicily, Punta Longa. SH:PLA_1 (S)	28,4	41	37	9,6	8,9	2,5	3	2	1,8	1,2	4	5,1	8,3	9,2	7
<i>B. hybridum</i>	Italy: Sicily, Santa Marina. SH:SMN_1 (S)	30,3	36	24	6,8	9,7	1,9	2	1,3	1,4	0,9	4	4,6	7,6	7,4	6
<i>B. hybridum</i>	Italy: Sicily, Cappella Palatina. SH:VCP_1 (S)	24,6	32	39	9,4	12	2,3	3	2,4	1,7	1,1	5,4	5	7,6	8,6	6
<i>B. hybridum</i>	Leibanon. INRA-V:Aja-1 (S)	33	38	44	7	8,6	2,7	3	2,2	1,8	1,1	7,1	5,9	11	13	7
<i>B. hybridum</i>	Morocco: E Morocco, Oujda, , near Argelia. USDA:PL253334 (S)	34,4	38	37	6,6	12	3,2	4	2,8	2,5	1,8	6,6	6,3	10	12	7
<i>B. hybridum</i>	Pakistan: Khyber Pakhtunkhwa, Pabbi. USDA:PL250647 (S)	33,2	43	43	4,8	10	2,8	3	3	2	1,4	6,4	6	9,6	13	7
<i>B. hybridum</i>	Portugal: Tras-os-Montes,Mogadouro. AM:Mog (S)	31,6	37	40	4	4,5	2,1	4	2	2,6	1,7	10	3	5,3	16	7
<i>B. hybridum</i>	South Africa: E Cape, near Darling. USDA:PL208216 (S)	29,2	40	45	8	11	2,9	3	3,8	1,5	1	5,6	5,2	8,4	8,6	6
<i>B. hybridum</i>	Spain: Alicante, Xávea, playa La Barraca. PC:B101 (S)	32	37	15	6,4	5,4	1,7	3	2,2	1,3	0,8	5,5	6,5	7,6	9,9	7
<i>B. hybridum</i>	Spain: Almeria, Cabo de Gata, Playa de Genoveses. PC:B103 (W)	25	46	51	6	10	2,2	4	3,8	1,8	1,1	7,9	4,7	8,1	11	7
<i>B. hybridum</i>	Spain: Almeria, Cabo de Gata, Playa de Genoveses. PC:B104 (W)	29,2	36	43	4,4	10	2,5	4	3,4	1,9	1,3	7,3	4,8	9,1	10	7
<i>B. hybridum</i>	Spain:Almeria, Cabo de Gata, Playa de Genoveses. PC:B105 (W)	26,6	36	39	4	8,6	2,8	3	2,6	2,3	1,7	7,4	4,7	11	14	7
<i>B. hybridum</i>	Spain: Almeria, Cabo de Gata, Playa de Genoveses. PC:B106 (W)	33,7	38	43	6,3	10	2	4	3,1	2,1	1,5	7,5	4,8	9,1	11	7
<i>B. hybridum</i>	Spain: Almeria, Cabo de Gata, Playa de Genoveses. PC:B107 (W)	29,8	37	41	5,2	9,5	2,3	3	3	1,7	1,1	5,8	4,8	9,3	10	7
<i>B. hybridum</i>	Spain: Barcelona, Montjuich.PC&LM:B37 (W)	30	36	34	3	5,8	2	6	4	2,5	1,5	11	7,5	9	15	6
<i>B. hybridum</i>	Spain: Canarias, Fuerteventura, Puerto del Rosario.INIA-CRF:BGE044244 (S)	29,8	46	44	3	5,9	3,1	6	3	3,2	2,3	13	7,4	11	9,3	8
<i>B. hybridum</i>	Spain: Canarias, Fuerteventura, Betancuria. INIA-CRF:BGE044245 (S)	31	48	39	2,5	4,6	3,2	6	2,5	4	3	13	8,9	12	8,3	8

<i>B. hybridum</i>	Spain: Canarias, Fuerteventura,, Betancuria.INIA-CRF:BGE044246 (S)	33,4	48	47	3	4,8	1,9	6	3	2,9	1,9	11	7,7	11	9,1	5
<i>B. hybridum</i>	Spain: Canarias, Gomera, Vallehermoso, Tamargada. INIA-CRF:BGE044242 (S)	29,2	38	65	4	5,4	2	5	4,5	2,9	1,3	12	6,7	9,2	15	7
<i>B. hybridum</i>	Spain: Canarias, Gomera, Arure_Vallehermoso 3km NE. INIA-CRF:BGE044243 (S)	25	31	48	3	6,1	3,4	4	2	2,3	1,7	12	7,5	8,9	13	8
<i>B. hybridum</i>	Spain: Canarias, Lanzarote, Tegui. INIA-CRF:BGE044247 (S)	30,4	48	48	3	7,5	2	7	3	2,9	2,1	11	8,2	12	9,6	8
<i>B. hybridum</i>	Spain: Canarias, Lanzarote, Tegui.INIA-CRF:BGE044248 (S)	29,2	29	40	3,5	6,5	1,9	5	3,5	2,7	1,7	9,5	8,6	10	18	7
<i>B. hybridum</i>	Spain: Canarias, Tenerife, Buenavista del Norte, Las Canales. INIA-CRF:BGE044238 (S)	29,4	46	40	5,5	12	2,6	5	3,5	2,5	1,7	12	6,8	8,9	17	8
<i>B. hybridum</i>	Spain: Canarias, Tenerife, Buenavista del Norte, Mascas. INIA-CRF:BGE044239 (S)	26,2	44	45	3	7,3	3,6	5	3	2,6	2,1	11	7,5	10	7,6	8
<i>B. hybridum</i>	Spain: Canarias, Tenerife, El Rosario, La esperanza. INIA-CRF:BGE044240 (S)	24,3	43	42	3	6	3,1	4	3,5	2,3	1,7	8	6,8	9,4	12	9
<i>B. hybridum</i>	Spain: Ciudad Real,Puertollano, sierra de Puertollano, Cerro del Castellar. HS:87745-2 (S)	29,2	34	39	8,3	8,9	2	4	2	2,6	2	9,5	8,7	8,7	9,9	7
<i>B. hybridum</i>	Spain: Cordoba, Guadalcazar. INRA:Esp1 (S)	29,2	45	76	5	4,7	2,1	4	2,7	2,9	2,2	13	6,4	8,8	11	7
<i>B. hybridum</i>	Spain: Córdoba, Córdoba.176F** (I)	31,6	43	57	8	10	2,6	4	2,8	1,8	1,4	6,8	8,1	9,9	13	7
<i>B. hybridum</i>	Spain: Girona, Cap de Lladró. PC&LM:B36 (W)	31,5	32	25	4	3,8	1,5	4	3	1,7	1,3	9	8	8	13	6
<i>B. hybridum</i>	Spain: Granada, Sierra Huetor, Puerto de la Mora. PC:B112 (W)	30,6	38	19	7,6	7,2	2,6	3	1,8	2,3	1,6	8	5,7	8,7	11	6
<i>B. hybridum</i>	Spain: Granada, Sierra Huetor, Puerto de la Mora. PC:B130 (W)	29,3	36	12	3	1,7	1,6	2	1,3	1,6	1	5	3,9	8,6	10	7
<i>B. hybridum</i>	Spain: Huelva. 333F** (I)	21,5	40	60	8	6,5	2,5	6	4	2,5	2	10	8,4	10	16	6
<i>B. hybridum</i>	Spain: Huesca, Graus. PC&LM:B16 (W)	31	32	20	3,3	4,4	1,9	4	2,3	2,4	1,2	11	7,8	8,8	16	6
<i>B. hybridum</i>	Spain: Jaen, Sierra Segura, Cortijos Nuevos. PC:B113 (W)	25,2	27	33	5,2	9,1	2,7	4	3,6	2,2	1,6	5,2	6,9	12	14	6
<i>B. hybridum</i>	Spain: Jaen, Sierra Segura, Cortijos Nuevos, Finca La Vaquilla. PC:B114 (W)	30,2	32	45	7,4	7,1	2,6	3	1,4	2,9	2,2	11	6,5	11	15	7
<i>B. hybridum</i>	Spain: Jaén,Sierra Cazorla, Cazorla, Paso del Aire, Mirador de las Palomas. PC:B118 (W)	32	33	44	10,4	9,9	2	3	3	1,7	1,2	5,4	4,2	8	9,9	7
<i>B. hybridum</i>	Spain: Jaén, Hinojares, Hinojares 2. PC:B122 (W)	28,6	32	43	4,4	8,5	2,8	3	2,8	2,4	1,8	6,9	6,7	11	11	7
<i>B. hybridum</i>	Spain: Jaén, Fontanar, Los Cotos2. PC:B124 (W)	27	31	23	4	2,8	1,8	4	3	2,6	2	12	5,7	7,8	14	7
<i>B. hybridum</i>	Spain: Jaén, Fontanar - Los cotos, río Guadiana Menor. PC:B125 (W)	28,2	32	40	3,8	10	2,9	4	3,4	2,4	1,6	5,9	6,4	12	14	8
<i>B. hybridum</i>	Spain: Jaen, Universidad de Jaen. PC:B131 (W)	27,8	36	38	6	5,3	2,3	3	2,4	2	1,4	7,7	5,5	11	14	7

<i>B. hybridum</i>	Spain: Jaén, Sra de Jaen, Salto de la Cabra. PC:B132 (W)	30,4	37	36	8,8	8,1	2,2	3	1,8	2,4	1,8	7,4	5,2	9,2	10	6
<i>B. hybridum</i>	Spain: Jaen, Sra de Jaen, Quiebrajano. PC:B133 (W)	32,8	36	26	8,6	4,4	2,3	3	1,6	2,5	1,8	7,6	5,2	8,7	11	6
<i>B. hybridum</i>	Spain: Jaen, Cazorla, Sierra Quesada, Tiscar, Monasterio de Ntra Sra de Tiscar. PC:B119bis (W)	28,8	39	35	10	8,4	2,8	3	2,6	1,8	1,2	5,5	4,9	8,8	8,3	6
<i>B. hybridum</i>	Spain: Jaen. 137F** (I)	28,6	45	73	9,3	9,3	2,2	7	4,8	2,5	2	12	8,8	10	15	8
<i>B. hybridum</i>	Spain: Málaga, INRA-V:Estepona-0 (S)	25,4	37	37	8	11	3,1	3	2,6	1,9	1,4	5,3	5,3	8,3	9,5	6
<i>B. hybridum</i>	Spain: Málaga, Abdalajis.260F** (I)	28,5	32	73	8	3,2	2,3	4	3	2,5	2	8,5	8,6	8,7	13	7
<i>B. hybridum</i>	Spain: Mallorca, Pto Pollensa. DL&PC:B600 (W)	25,8	34	33	4,5	4,6	2,5	3	2	3	1,7	9	8,2	9	13	7
<i>B. hybridum</i>	Spain: Mallorca, Pto Pollensa, Formentor. DL&PC:B681 (W)	31	41	4,5	1,7	1,2	1	2	1	1,7	0,9	8	7,5	5,1	8,7	6
<i>B. hybridum</i>	Spain: Mallorca, Pto Pollensa, Mal Pas. DL&PC:B701 (W)	30,5	40	12	4	3,7	1,4	3	2	2	1,4	9,7	3,5	3,1	8,3	6
<i>B. hybridum</i>	Spain: Mallorca, Pto Pollensa, St Vicent. DL&PC:B731 (W)	26,4	37	25	8	4,1	1,9	2	1,3	2	1,3	8	4,2	3,6	14	6
<i>B. hybridum</i>	Spain: Mallorca, Pollensa-Soller, Temenia. DL&PC:B741 (W)	27	35	20	4	2,9	1,5	3	2,7	1,9	1,3	9,3	5,5	7,6	13	6
<i>B. hybridum</i>	Spain: Mallorca, Escorca, Tossels Cuvert. DL&PC:B781 (W)	29,4	39	9,7	3	4	1,5	2	1,3	1,6	1,1	7,7	5,7	7,8	12	7
<i>B. hybridum</i>	Spain: Mallorca, Escorca, Pico del Tossel. DL&PC:B791 (W)	30	46	7,6	2,5	1,7	1,3	2	1,3	1,5	1,1	8	5	6,7	9,1	6
<i>B. hybridum</i>	Spain: Mallorca, Escorca, Cuvert. DL&PC:B811 (W)	31,5	45	15	2,5	3,2	1,5	2	1	2,1	1,5	10	6,3	8	11	7
<i>B. hybridum</i>	Spain: Mallorca, Deia. DL&PC:B831 (W)	29	34	14	4,3	3,8	1,8	3	2	2,1	1,4	10	4,9	4,6	10	6
<i>B. hybridum</i>	Spain: Mallorca, Escorca, Sa Calobra. DL&PC:B851 (W)	25	43	20	4	5	1,8	2	2	2,4	1,4	11	3,9	4,2	12	6
<i>B. hybridum</i>	Spain: Mallorca, Paguera, Cala Fornell. DL&PC:B871 (W)	29	36	18	3	3,4	1,3	4	3,3	1,8	1,4	10	6,5	7,9	11	6
<i>B. hybridum</i>	Spain: Mallorca, Valldemosa, Sa Marina. DL&PC:B931 (W)	27,6	39	10	3,4	2,6	1,7	2	1,2	2	1,4	8,6	6,2	7,8	11	5
<i>B. hybridum</i>	Spain: Mallorca, Lluçmajor, Algaida. DL&PC:B1101 (W)	28,8	41	35	3,7	2,6	1,6	5	3	3,5	2,5	16	6,9	9,2	9	7
<i>B. hybridum</i>	Spain: Menorca, Foruella, Torre de Foruella. DL&PC:B1001 (W)	28	37	20	4,7	3	1,3	3	2,3	1,7	1,1	9,3	4,5	6,4	9,7	6
<i>B. hybridum</i>	Spain: Menorca, Ciutadella, Port. DL&PC:B1011 (W)	28,1	36	29	4	6,3	2,1	4	2,8	2,2	1,6	9,8	6,2	8,8	14	7
<i>B. hybridum</i>	Spain: Murcia,España. AM:España (S)	34,8	43	49	6,5	2,8	0,8	4	2,7	2,5	1,6	9	4,3	8,1	16	8
<i>B. hybridum</i>	Spain: Murcia, Alhama de Murcia, Srra España1. PC:B137 (W)	28,4	41	34	4,6	11	2,3	4	3	2,1	1,4	8,8	5,8	11	12	7
<i>B. hybridum</i>	Spain: Murcia, Alhama de Murcia, Srra España2. PC:B138 (W)	33	38	71	7,4	11	3,1	4	3,8	2,2	1,5	7,6	5,2	8,1	12	7
<i>B. hybridum</i>	Spain: Murcia, Alhama de Murcia, Srra España3. PC:B139 (W)	29,4	41	43	5,4	9,5	2,2	3	2,6	2	1,3	8,3	5,5	9,9	9,9	7
<i>B. hybridum</i>	Spain: Tarragona, Amposta. PC&LM:B39 (W)	29,3	39	24	3,3	3,4	1,6	4	3,3	1,8	1,4	8,3	7,3	7,3	14	6
<i>B. hybridum</i>	Spain: Tarragona, Poble Nou del Delta. PC&LM:B40 (W)	32,6	38	18	2	2,9	2	5	2,8	2,8	1,5	10	8,8	9,6	14	7

<i>B. hybridum</i>	Spain: Teruel, Calaceite. PC&LM:B41 (W)	26,2	32	18	2	2,1	1,7	4	2,4	2,3	1,1	9,2	5,6	7	7,6	6
<i>B. hybridum</i>	Spain: Zaragoza, Belchite. PC&LM:B42 (W)	31,7	39	12	2,3	2,1	1,6	3	2,3	1,8	1,1	10	5,3	7,3	8,3	6
<i>B. hybridum</i>	Uruguay. USDA:PL372187 (S)	27	36	42	7	11	3,6	4	4	2	1,4	6,8	4,3	8,4	10	6

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**Table S2.** Morphological variables used in the phenotypic analysis. Results of the Kolmogorov-Smirnov normality test. Significance: \*\*\*:  $p < 0.001$ ; \*\*:  $p < 0.01$ ; \*:  $p < 0.05$ ; n.s.: non-significant.

Trait	Code	Statistic
Leaf guard cell length in the stomata (mm)	LGCL	0.076*
Pollen grain length (mm)	PGL	0.074*
Plant height (cm)	H	0.074*
Number of nodes of tallest culm	NNTC	0.113***
Second leaf length from the base of the plant (cm)	SLL	0.107***
Second leaf width (mm)	SLW	0.081**
Inflorescence length (cm)	IL	0.082**
Number of spikelets per inflorescence	NSI	0.099***
Spikelet total length, without awns (cm)	SLA	0.094***
Spikelet length from the base to the apex of the fourth lemma, without awns (cm)	SLB	0.095***
Number of flowers per inflorescence	NFS	0.064 <sup>n.s.</sup>
Upper glume length (mm)	UGL	0.060 <sup>n.s.</sup>
Lemma length from the basal floret (mm)	LL	0.050 <sup>n.s.</sup>
Awn length, the longest within the spikelet (mm)	AL	0.080*
Caryopsis length (mm)	CL	0.032 <sup>n.s.</sup>

**Table S3.** Pairwise Spearman correlation coefficients between phenotypic traits (averaged values) analyzed in wild individuals of *B. distachyon*, *B. stacei* and *B. hybridum*. Significant correlation values ( $p < 0.001$ ) are highlighted in bold.

	LGCL	PGL	H	NNTC	SLL	SLW	IL	NSI	SLA	SLB	NFI	UGL	LL	AL	CL
LGCL															
PGL	<b>0.54</b>														
H	0.14	0.21													
NNTC	0.11	0.16	<b>0.52</b>												
SLL	0.33	0.26	<b>0.66</b>	<b>0.56</b>											
SLW	0.16	0.16	<b>0.53</b>	0.33	<b>0.69</b>										
IL	0.15	0.20	<b>0.70</b>	0.09	0.37	0.40									
NSI	0.03	0.01	<b>0.65</b>	0.18	0.46	0.38	<b>0.75</b>								
SLA	0.25	0.38	0.41	-0.02	0.18	0.34	<b>0.65</b>	0.20							
SLB	0.19	0.37	0.39	0.03	0.15	0.29	0.58	0.16	<b>0.89</b>						
NFI	0.01	0.22	0.04	-0.38	-0.31	-0.13	0.40	0.07	<b>0.62</b>	<b>0.59</b>					
UGL	-0.11	-0.04	0.07	-0.19	-0.15	0.10	0.29	0.15	0.30	0.32	0.35				
LL	0.24	0.24	0.49	0.14	0.45	0.57	0.55	0.28	<b>0.59</b>	<b>0.56</b>	0.07	0.32			
AL	-0.03	0.04	0.23	0.06	0.08	0.19	0.40	0.21	0.40	0.39	0.24	0.34	0.42		
CL	0.37	0.44	0.50	0.17	0.40	0.41	0.52	0.24	0.58	0.55	0.12	0.12	0.64	0.38	

**Table S4.** Loadings of phenotypic traits onto the first PCA components. Highest contributions of characters to each component are highlighted in bold.

Phenotypic trait	C 1	C 2	C 3
LGCL	0.043	0.451	<b>-0.608</b>
PGL	0.092	<b>0.876</b>	0.301
H	<b>0.982</b>	-0.112	0.042
NNTC	0.061	-0.004	-0.217
SLL	0.114	0.054	-0.465
SLW	0.019	0.004	-0.037
IL	0.053	0.002	0.091
NSS	0.034	-0.020	-0.002
SLA	0.013	0.028	0.050
SLB	0.010	0.022	0.049
NFI	0.014	0.091	0.421
UGL	0.010	-0.010	0.147
LL	0.046	0.030	0.006
AL	0.041	-0.001	0.246
CL	0.028	0.055	0.010

**Table S5.** Contribution of phenotypic variables to the first canonical discriminant functions obtained for the two data sets of individuals of *B. distachyon*, *B. stacei* and *B. hybridum* analyzed: W (174 wild populations data set). I + W (6 inbred lines + 174 wild populations data set). Variables that contributed more to the functions are highlighted in bold.

W			I+W		
	1	2		1	2
<b>LGCL</b>	<b>0.545*</b>	0.391	<b>LGCL</b>	<b>0.564*</b>	0.375
<b>CL</b>	<b>0.475*</b>	-0.066	<b>CL</b>	<b>0.458*</b>	-0.051
<b>PGL</b>	<b>0.424*</b>	0.249	<b>PGL</b>	<b>0.442*</b>	0.243
<b>SLL</b>	0.347*	0.019	<b>SLA</b>	0.351*	-0.053
<b>SLA</b>	0.344*	-0.041	<b>SLL</b>	0.339*	0.006
<b>LL</b>	0.298*	-0.152	<b>LL</b>	0.303*	-0.143
<b>H</b>	0.293*	-0.139	<b>H</b>	0.298*	-0.14
<b>SLB</b>	0.272*	-0.097	<b>SLB</b>	0.276*	-0.101
<b>SLW</b>	0.257*	-0.153	<b>SLW</b>	0.238*	-0.174
<b>NNTC</b>	0.125*	0.117	<b>NNTC</b>	0.129*	0.119
<b>IL</b>	0.396	<b>-0.519*</b>	<b>IL</b>	0.391	<b>-0.528*</b>
<b>NSI</b>	0.177	-0.188*	<b>NSI</b>	0.17	-0.178*
<b>AL</b>	0.135	-0.163*	<b>AL</b>	0.128	-0.143*
<b>UGL</b>	-0.001	0.058*	<b>UGL</b>	-0.003	0.069*
<b>NFI</b>	0.055	-0.057*	<b>NFI</b>	0.06	-0.064*



**Table S6.** Contribution of phenotypic variables to the first canonical discriminant functions obtained for individuals from 41 wild populations of *B. distachyon*. Variables that contribute more to the functions are highlighted in bold.

	Function	
	1	2
H	<b>0.565(*)</b>	0.191
LGCL	-0.108(*)	-0.063
UGL	0.002	<b>0.656(*)</b>
AL	-0.048	<b>0.464(*)</b>
NNTC	0.317	<b>-0.461(*)</b>
NSS	0.154	0.411(*)
PGL	0.022	0.258(*)
LL	0.022	0.158(*)
CL	0.057	-0.026
SLB	0.045	0.262
SLW	0.049	0.140
SLL	0.286	-0.022
IL	0.109	0.451
NFI	-0.064	0.384
SLA	-0.047	0.221

**Table S7.** Contribution of phenotypic variables to the first canonical discriminant functions obtained for individuals from 29 wild populations of *B. stacei*. Variables that contribute more to the functions are highlighted in bold.

	Function						
	1	2	3	4	5	6	7
IL	<b>-0.111(*)</b>	0.019	0.076	-0.052	0.075	0.071	0.034
SLB	0.027	<b>0.185(*)</b>	-0.093	0.025	-0.032	0.069	0.046
SLA	0.015	<b>0.167(*)</b>	-0.107	-0.133	0.051	0.096	0.011
NNTC	-0.083	0.021	<b>0.328(*)</b>	0.229	0.020	0.060	0.098
AL	-0.008	0.095	<b>0.266(*)</b>	0.095	-0.034	0.142	0.241
UGL	-0.014	-0.018	0.135	<b>0.268(*)</b>	-0.081	-0.085	-0.111
H	-0.217	-0.030	0.130	-0.123	<b>0.493(*)</b>	0.228	0.143
NSS	-0.092	-0.070	0.131	-0.089	<b>0.239(*)</b>	-0.138	-0.030
SLL	-0.097	-0.102	0.122	-0.155	0.085	<b>0.630(*)</b>	-0.123
CL	0.020	0.142	-0.005	0.291	0.229	<b>0.305(*)</b>	-0.005
PGL	0.129	0.200	0.023	0.233	0.128	<b>0.303(*)</b>	0.294
LGCL	0.054	0.083	-0.137	0.008	-0.100	<b>-0.180(*)</b>	0.108
SLW	-0.028	0.011	0.121	-0.156	0.060	0.234	<b>-0.530(*)</b>
NFI	0.055	0.094	-0.130	-0.073	0.042	0.027	<b>0.267(*)</b>
LL	-0.022	0.104	0.190	-0.208	0.205	-0.017	<b>-0.209(*)</b>

**Table S8.** Contribution of phenotypic variables to the first canonical discriminant functions obtained for individuals from 89 wild populations of *B. hybridum*. Variables that contribute more to the functions are highlighted in bold.

	Function				
	1	2	3	4	5
H	<b>0.508(*)</b>	-0.219	-0.431	0.009	0.074
SLW	<b>0.440(*)</b>	-0.053	0.065	0.053	0.094
CL	<b>0.402(*)</b>	0.120	0.174	0.136	0.142
NNTC	0.172	<b>-0.571(*)</b>	-0.026	0.006	0.232
UGL	0.141	<b>0.486(*)</b>	0.038	-0.203	-0.136
NFI	-0.086	<b>0.420(*)</b>	-0.195	0.152	-0.245
SLL	0.337	<b>-0.388(*)</b>	-0.201	-0.043	0.020
IL	0.291	0.279	<b>-0.444(*)</b>	-0.275	-0.415
NSS	0.168	-0.069	<b>-0.394(*)</b>	-0.342	0.010
AL	0.114	0.134	<b>0.343(*)</b>	-0.158	0.112
PGL	0.129	0.252	-0.199	<b>0.527(*)</b>	-0.033
LGCL	0.008	-0.085	-0.133	<b>-0.286(*)</b>	0.071
SLB	0.159	0.190	-0.065	0.342	<b>-0.590(*)</b>
LL	0.504	0.094	0.390	-0.138	<b>-0.530(*)</b>
SLA	0.145	0.278	-0.118	0.174	<b>-0.500(*)</b>

**Table S9.** Annotation of 434 ‘explanatory’ nominal mass fingerprint signals by targeted accurate  $m/z$  data analysis and MZedDB database search at MSI level of identification 2. The  $m/z$  signal with duplicate annotations, i.e., other adducts or isotopes of same metabolite, are indicated as plain (un-bold) text .

PW Class	Nominal $m/z$	Ionisation Mode	Accurate $m/z$	Max. Intensity	Theoretical $m/z$	Molecular Formula	Putative Ionization Product	Adduct	PPM Error
2N=10~2N=30	71.09	Negative	<b>71.01350</b>	<b>88389</b>	<b>71.01385</b>	<b>C3H4O2</b>	<b>Propenoate</b>	<b>[M-H]1-</b>	<b>-4.97</b>
E137F00h~E484F00h	72.09	Negative	72.00872	723	72.00910	C2H3NO2	Imino glycine	[M-H]1-	-5.36
			<b>72.01682</b>	<b>1230</b>	-	-	<b>Unknown</b>	-	-
E160F00h~E485F00h	73.09	Negative	72.99276	17632	72.99312	C2H2O3	Glyoxylate	[M-H]1-	-4.86
			<b>73.02914</b>	<b>2623</b>	<b>73.02950</b>	<b>C3H6O2</b>	<b>Propanoate</b>	<b>[M-H]1-</b>	<b>-5.00</b>
2N=10~2N=30	87.09	Negative	<b>87.00848</b>	<b>97834</b>	<b>87.00877</b>	<b>C3H4O3</b>	<b>Pyruvate</b>	<b>[M-H]1-</b>	<b>-3.26</b>
E115F00h~E176F00h	88.09	Negative	<b>88.04009</b>	<b>32696</b>	<b>88.04040</b>	<b>C3H7NO2</b>	<b>Sarcosine</b>	<b>[M-H]1-</b>	<b>-3.52</b>
E129F00h~E137F00h	89.09	Negative	<b>88.98781</b>	<b>29000</b>	<b>88.98803</b>	<b>C2H2O4</b>	<b>Oxalate</b>	<b>[M-H]1-</b>	<b>-2.56</b>
			<b>89.02416</b>	<b>83599</b>	<b>89.02442</b>	<b>C3H6O3</b>	<b>Lactate</b>	<b>[M-H]1-</b>	<b>-2.90</b>
2N=10~2N=20	91.09	Negative	90.99288	2589	90.99312	C7H4O6	Chelidonate	[M-2H]2-	-2.60
E137F00h~E160F00h	100	Negative	<b>100.04012</b>	<b>741</b>	<b>100.04040</b>	<b>C4H7NO2</b>	<b>Homoserine Lactone</b>	<b>[M-H]1-</b>	<b>-2.82</b>
			100.07653	483	100.07679	C5H11NO	5-Amino pentanal	[M-H]1-	-2.57
E137F00h~E485F00h	101.09	Negative	<b>101.00599</b>	<b>1569</b>	-	-	<b>Unknown</b>	-	-
			<b>101.02414</b>	<b>13018</b>	<b>101.02442</b>	<b>C4H6O3</b>	<b>Oxo butanoate</b>	<b>[M-H]1-</b>	<b>-2.75</b>
E115F00h~E260F00h	102.09	Negative	<b>102.05576</b>	<b>34229</b>	<b>102.05605</b>	<b>C4H9NO2</b>	<b><math>\gamma</math>-Amino butyrate</b>	<b>[M-H]1-</b>	<b>-2.86</b>
2N=10~2N=30	103.09	Negative	<b>103.00346</b>	<b>5226</b>	<b>103.00368</b>	<b>C3H4O4</b>	<b>Hydroxy pyruvate</b>	<b>[M-H]1-</b>	<b>-2.16</b>
			<b>103.03980</b>	<b>1511</b>	<b>103.04007</b>	<b>C4H8O3</b>	<b>Hydroxy butyrate</b>	<b>[M-H]1-</b>	<b>-2.60</b>
E114F00h~E115F00h	104.09	Negative	104.03508	18939	104.03532	C3H7NO3	Serine	[M-H]1-	-2.28
2N=10~2N=20	105.09	Negative	<b>105.01908</b>	<b>34360</b>	<b>105.01933</b>	<b>C3H6O4</b>	<b>Glycerate</b>	<b>[M-H]1-</b>	<b>-2.41</b>
E114F00h~E480F00h	107.09	Negative	<b>107.05008</b>	<b>502</b>	<b>107.05024</b>	<b>C7H8O</b>	<b><i>p</i>-Cresol</b>	<b>[M-H]1-</b>	<b>-1.48</b>
E114F00h~E176F00h	116.09	Negative	<b>116.00687</b>	<b>4668</b>	-	-	<b>Unknown</b>	-	-
			116.07155	1962	116.07170	C5H11NO2	Valine	[M-H]1-	-1.31
E137F00h~E333F00h	117	Negative	<b>117.01909</b>	<b>11733</b>	<b>117.01933</b>	<b>C4H6O4</b>	<b>Succinate</b>	<b>[M-H]1-</b>	<b>-2.08</b>
2N=20~2N=30	118.09	Negative	118.05079	19614	118.05097	C4H9NO3	Threonine	[M-H]1-	-1.50
E137F00h~E485F00h	119	Negative	<b>118.93055</b>	<b>1684</b>	<b>118.93059</b>	<b>H3O3P</b>	<b>Phosphonate</b>	<b>[M+K-2H]1-</b>	<b>-0.32</b>
			118.94190	5134	118.94205	H2O4S	Sulfate	[M+Na-2H]1-	-1.28

2N=10~2N=20	121	Negative	119.03482	1284	119.03498	C4H8O4	Erythrose / Threose	[M-H]1-	-1.37
			119.05007	3481	119.05024	C8H8O	4-Hydroxy styrene	[M-H]1-	-1.41
			120.99634	797	120.99649	C3H6O3S	3-Mercapto lactate	[M-H]1-	-1.26
E115F00h~E162F00h	123	Negative	121.02928	2939	121.02950	C7H6O2	Benzoate	[M-H]1-	-1.84
			121.06566	716	121.06589	C8H10O	2,4-Dimethyl phenol	[M-H]1-	-1.88
			123.00857	737	123.00877	C6H4O3	2-Hydroxy-1,4-Benzoquinone	[M-H]1-	-1.61
E114F00h~E137F00h	124	Negative	123.04494	524	123.04515	C7H8O2	3-Hydroxybenzyl alcohol	[M-H]1-	-1.73
			124.00726	428	124.00739	C2H7NO3S	Taurine	[M-H]1-	-1.06
			124.01654	846	124.01691	C2H8NO3P	2-Aminoethyl phosphonate	[M-H]1-	-2.94
E129F00h~E137F00h	125	Negative	124.04027	513	124.04040	C6H7NO2	3,6-Dihydro nicotinate	[M-H]1-	-1.06
			124.05179	430	124.05164	C5H7N3O	5-Methyl cytosine	[M-H]1-	1.25
			125.02426	1306	125.02442	C6H6O3	Pyrogallol	[M-H]1-	-1.26
E115F00h~E176F00h	126	Negative	125.06060	569	125.06080	C7H10O2	3-Heptynoate	[M-H]1-	-1.62
			126.01957	545	126.01967	C5H5NO3	2,3,6-Trihydroxy pyridine	[M-H]1-	-0.77
			129.09	2905	-	-	Unknown	-	-
2N=10~2N=20	129.09	Negative	129.01918	28289	129.01933	C5H6O4	Glutaconate	[M-H]1-	-1.19
			129.03854	1499	-	-	Unknown	-	-
			129.05555	1086	129.05572	C6H10O3	5-Oxo hexanoate	[M-H]1-	-1.30
E137F00h~E160F00h	132.09	Negative	132.03001	133167	132.03023	C4H7NO4	Aspartate	[M-H]1-	-1.68
			133.01404	689515	133.01425	C4H6O5	Malate	[M-H]1-	-1.56
			134.01745	26979	-	-	13C Isotope of Malate	[M-H]1-	-
2N=10~2N=30	134.09	Negative	134.04706	1023	134.04722	C5H5N5	Adenine	[M-H]1-	-1.18
			134.91583	11326	134.91599	H2O4S	Sulfate	[M+K-2H]1-	-1.18
			135.02970	55697	135.02990	C4H8O5	Threonate	[M-H]1-	-1.47
2N=10~2N=20	136	Negative	135.92070	23574	-	-	Unknown	-	-
			136.01640	1971	-	-	Unknown	-	-
			136.03305	1436	-	-	Unknown	-	-
E129F00h~E137F00h	138	Negative	136.04023	694	136.04040	C7H7NO2	p-Amino benzoate	[M-H]1-	-1.26
			136.07649	631	136.07679	C8H11NO	Tyramine	[M-H]1-	-2.18
			137.99568	2854	137.99628	C4H7NO2	Aceto acetamide	[M+K-2H]1-	-4.38
2N=10~2N=20	139	Negative	138.01932	463	138.01967	C6H5NO3	6-Hydroxy nicotinate	[M-H]1-	-2.51
			138.05577	1700	138.05605	C7H9NO2	Trigonelline	[M-H]1-	-2.04
			138.97999	1178	138.98019	C2H5O5P	Acetyl Phosphate	[M-H]1-	-1.41

2N=10~2N=30	142	Negative	139.00351	29424	139.00368	C3H2O2	Propynoate	[2M-H]1-	-1.24
			139.03986	712	139.04007	C7H8O3	4-Hydroxy methyl catechol	[M-H]1-	-1.50
2N=10~2N=20	143.09	Negative	139.07618	668	139.07645	C8H12O2	2-Octynoate	[M-H]1-	-1.96
			142.05078	826	-	-	Unknown	-	-
E114F00h~E137F00h	144.09	Negative	142.97488	1195	142.97522	C3H6O4	Glycerate	[M+K-2H]1-	-2.34
			142.99836	18394	-	-	Unknown	-	-
E137F00h~E160F00h	145.09	Negative	143.03478	1303	143.03498	C6H8O4	3-Methyl glutaconate	[M-H]1-	1.21
			143.07116	956	143.07137	C7H12O3	n-Valeryl acetate	[M-H]1-	2.71
2N=10~2N=30	146.09	Negative	143.10763	816	143.10775	C8H16O2	Caprylate	[M-H]1-	-0.86
			144.03006	577	144.03023	C5H7NO4	2-Oxo glutaramate	[M-H]1-	-1.19
2N=20~2N=30	147	Negative	144.06638	751	144.06662	C6H11NO3	5-Hydroxy-pipecolate	[M-H]1-	-1.65
			145.01397	5041	145.01425	C5H6O5	Oxoglutarate	[M-H]1-	-1.92
Pilar analysis	148.09	Negative	145.05044	678	145.05063	C6H10O4	Adipate	[M-H]1-	-1.33
			145.06165	41934	145.06187	C5H10N2O3	Alanyl glycine	[M-H]1-	-1.49
2N=10~2N=20	151	Negative	145.94952	9574	-	-	Unknown	-	-
			146.04567	223467	146.04588	C5H9NO4	3-Methyl aspartate	[M-H]1-	-1.45
2N=20~2N=30	152.09	Negative	147.02966	10402	147.02990	C5H8O5	Hydroxy glutarate	[M-H]1-	1.07
			147.04507	511	147.04515	C9H8O2	Cinnamate	[M-H]1-	-0.56
E333F00h~E484F00h	157	Negative	147.04901	6893	-	-	<sup>13</sup> C Isotope of 3-Methyl aspartate	[M-H]1-	-
			147.06601	1087	147.06628	C6H12O4	Mevalonate	[M-H]1-	-1.86
E137F00h~E480F00h	158	Negative	148.04368	587	148.04378	C5H11NO2S	L-Methionine	[M-H]1-	-0.65
			148.04985	1064	-	-	Unknown	-	-
2N=10~2N=20	152.09	Negative	151.03991	2748	151.04007	C8H8O3	Methyl salicylate / Vanillin	[M-H]1-	-1.05
			151.98280	2216	-	-	Unknown	-	-
E333F00h~E484F00h	157	Negative	152.01135	607	152.01193	C5H9NO2	Proline	[M+K-2H]1-	-3.84
			152.03502	547	152.03532	C7H7NO3	p-Amino salicylate	[M-H]1-	-1.95
E137F00h~E480F00h	158	Negative	152.04815	736	152.04838	C5H11NO2	Valine	[M+Cl]1-	-1.51
			152.07148	394	152.07170	C8H11NO2	Dopamine	[M-H]1-	-1.46
E137F00h~E480F00h	158	Negative	156.96285	1709	-	-	Unknown	-	-
			157.03637	1052	157.03671	C4H6N4O3	Allantoin	[M-H]1-	-2.19
E137F00h~E480F00h	158	Negative	157.05032	712	157.05063	C7H10O4	2-Isopropyl maleate	[M-H]1-	-1.99
			157.08683	709	157.08702	C8H14O3	2-Keto-N-caprylate	[M-H]1-	-1.20
E137F00h~E480F00h	158	Negative	158.04566	636	158.04588	C6H9NO4	N-Methyl-2-oxoglutaramate	[M-H]1-	-1.40

2N=10~2N=20	163	Negative	158.08205	512	158.08227	C7H13NO3	Valeryl glycine	[M-H]1-	-1.37
			162.93962	340840	-	-	Unknown	-	-
			163.03983	29190	163.04007	C9H8O3	<i>p</i> -Coumarate	[M-H]1-	-1.46
2N=20~2N=30	165	Negative	164.93770	18934	-	-	Unknown	-	-
			165.01912	2020	165.01933	C8H6O4	Phthalate	[M-H]1-	-1.29
			165.04032	18130	165.04046	C5H10O6	Xylonate	[M-H]1-	-0.87
2N=10~2N=20	168	Negative	165.05537	609	165.05572	C9H10O3	Caffeyl alcohol	[M-H]1-	-2.11
			168.02756	436	168.02783	C5H9NO4	Glutamate	[M+Na-2H]1-	-1.59
			168.04292	862	168.04323	C6H13NO2	Leucine / Isoleucine	[M+K-2H]1-	-1.87
E114F00h~E160F00h	171	Negative	170.96990	784	170.97013	C4H6O5	Malate	[M+K-2H]1-	-1.35
			171.00625	1079	171.00640	C3H9O6P	Glycerol 3-phosphate	[M-H]1-	-0.88
			171.02979	1336	171.02990	C7H8O5	3-Dehydro shikimate	[M-H]1-	-0.63
2N=10~2N=30	173.09	Negative	173.00904	29910	173.00916	C6H6O6	Dehydro ascorbate	[M-H]1-	-0.71
			173.04536	10551	173.04555	C7H10O5	Shikimate	[M-H]1-	-1.09
			173.08170	1955	173.08193	C8H14O4	Ethyl adipate	[M-H]1-	-1.35
2N=10~2N=30	174.09	Negative	174.04055	774	174.04080	C6H9NO5	N-Acetyl aspartate	[M-H]1-	-1.42
			174.04871	815	-	-	<sup>13</sup> C Isotope of Shikimate	[M-H]1-	-
			179.03491	2291	179.03498	C9H8O4	Caffeate	[M-H]1-	-0.41
2N=10~2N=30	179.09	Negative	179.05589	2268	179.05611	C6H12O6	Glucose	[M-H]1-	-1.25
			179.07106	457	179.07137	C10H12O3	Coniferyl Alcohol	[M-H]1-	-1.72
			181.01389	844	181.01425	C8H6O5	4-Hydroxy phthalate	[M-H]1-	-1.98
2N=10~2N=20	181.09	Negative	181.05044	755	181.05063	C9H10O4	Dihydro caffeate	[M-H]1-	-1.07
			181.93438	1582	-	-	Unknown	-	-
			181.94686	1114	-	-	Unknown	-	-
E115F00h~E160F00h	182.09	Negative	182.04557	1495	182.04588	C8H9NO4	5-Methoxy-3-hydroxyanthranilate	[M-H]1-	-1.71
			182.99338	9133	182.99351	C7H4O6	Chelidonate	[M-H]1-	-0.73
			183.02984	760	183.02990	C8H8O5	3-O-Methyl gallate	[M-H]1-	-0.32
2N=10~2N=30	183.09	Negative	183.10245	879	183.10267	C10H16O3	5-Oxo-7E-decenoate	[M-H]1-	-1.19
			183.99615	1148	-	-	Unknown	-	-
			184.00154	828	184.00165	C3H8NO6P	O-Phospho serine	[M-H]1-	-0.60
E114F00h~E137F00h	184.09	Negative	184.98556	21029	184.98567	C3H7O7P	3-Phospho glycerate	[M-H]1-	-0.57
			185.02198	657	185.02201	C9H8O3	<i>p</i> -Coumarate	[M+Na-2H]1-	-0.18
			185.02201	657	185.02201	C9H8O3	<i>p</i> -Coumarate	[M+Na-2H]1-	-0.18
E114F00h~E480F00h	185.09	Negative	187.09734	880	187.09758	C9H16O4	Azelaic acid	[M-H]1-	-1.30
2N=20~2N=30	187.09	Negative							

2N=10~2N=30	191.09	Negative	191.01952	493719	191.01973	C6H8O7	Citrate	[M-H]1-	-1.09
			191.05585	164745	191.05611	C7H12O6	Quinate	[M-H]1-	-1.38
2N=10~2N=30	192.09	Negative	192.02302	20039	-	-	13C Isotope of Citrate	[M-H]1-	-
			192.05922	6070	-	-	13C Isotope of Quinate	[M-H]1-	-
E137F00h~E480F00h	193.09	Negative	193.05061	13417	193.05063	C10H10O4	Ferulate	[M-H]1-	-0.12
2N=10~2N=30	194.09	Negative	194.04572	556	194.04588	C9H9NO4	Salicyl urate	[M-H]1-	-0.83
			194.05389	1033	194.05471	C6H11N3O3	2-Oxo arginine	[M+Na-2H]1-	-4.23
2N=10~2N=20	195.18	Negative	194.92756	669	194.92749	H2O4S	Sulfate	[2M-H]1-	0.35
			194.94634	3658	194.94652	H3O4P	Phosphorate	[2M-H]1-	-0.92
			195.05084	56939	195.05103	C6H12O7	Gluconate	[M-H]1-	-0.96
E176F00h~E480F00h	197.18	Negative	197.00909	915	197.00916	C8H6O6	3,4-Dihydroxy phthalate	[M-H]1-	-0.37
			197.02210	511	197.02217	C7H12O4	Pimelate	[M+K-2H]1-	-0.33
			197.04528	784	197.04555	C9H10O5	Syringate	[M-H]1-	-1.36
E114F00h~E115F00h	198	Negative	198.01718	1795	198.01730	C4H10NO6P	<i>o</i> -Phospho homoserine	[M-H]1-	-0.61
			198.04023	1046	198.04080	C8H9NO5	Clavulanate	[M-H]1-	-2.86
2N=20~2N=30	199.09	Negative	198.91787	577	198.91790	H4O7P2	Pyrophosphate	[M+Na-2H]1-	-0.15
			199.00123	975	199.00132	C4H9O7P	Erythrose-4-phosphate	[M-H]1-	-0.43
			199.13390	737	199.13397	C11H20O3	8-Propionyl caprylate	[M-H]1-	-0.34
2N=10~2N=20	201.09	Negative	201.00398	544	201.00408	C7H6O7	4-Oxalo mesaconate	[M-H]1-	-0.49
			201.04026	483	201.04046	C8H10O6	cis-(Homo) <sub>2</sub> aconitate	[M-H]1-	-1.01
			201.11298	603	201.11323	C10H18O4	Sebacate	[M-H]1-	-1.26
2N=10~2N=20	203.09	Negative	203.01972	55332	203.01973	C7H8O7	Oxaloglutarate	[M-H]1-	-0.04
2N=20~2N=30	204.09	Negative	204.02305	2530	-	-	13C Isotope of oxaloglutarate	[M-H]1-	-
			204.05154	774	204.05224	C8H15NOS2	Lipoamide	[M-H]1-	-3.41
2N=10~2N=20	207.09	Negative	206.94689	702	206.94663	C3H7O6P	Dihydroxyacetone phosphate	[M+K-2H]1-	1.24
			206.96775	616	206.96761	C3H7O7P	3-Phospho glycerate	[M+Na-2H]1-	0.67
			207.06622	912	207.06628	C11H12O4	Sinapaldehyde	[M-H]1-	-0.30
2N=10~2N=20	208.18	Negative	208.06149	752	208.06153	C10H11NO4	4-Hydroxyphenyl acetyl glycine	[M-H]1-	-0.20
E114F00h~E137F00h	210.09	Negative	209.94877	10220	-	-	Unknown	-	-
			210.04068	847	210.04080	C9H9NO5	DIMBOA	[M-H]1-	-0.56
E114F00h~E137F00h	211.09	Negative	211.00114	912	211.00143	C7H10O5	Shikimate	[M+K-2H]1-	-1.37
			211.02474	941	211.02481	C9H8O6	3-(2-Carboxyethenyl)-cis,cis-muconate	[M-H]1-	-0.35
			211.03746	544	211.03788	C7H12O5	2-Isopropyl malate	[M+Cl]1-	-1.97



E114F00h~E137F00h	212.09	Negative	211.99572	485	211.99657	C4H8NO7P	4-Phospho aspartate	[M-H]1-	-3.99
			212.05646	464	212.05645	C9H11NO5	N,N-Dihydroxy tyrosine	[M-H]1-	0.06
E137F00h~E162F00h	213.09	Negative	213.01704	859	213.01697	C5H11O7P	Deoxyribose 5-phosphate	[M-H]1-	0.35
			213.04049	812	213.04046	C9H10O6	2-Hydroxy-6-oxonona-2,4-diene-1,9-dioate	[M-H]1-	0.13
			213.14955	566	213.14962	C12H22O3	4-Keto laurate	[M-H]1-	-0.32
E114F00h~E115F00h	215.09	Negative	215.03268	1358	215.03279	C6H12O6	Glucose	[M+Cl]1-	-0.52
E114F00h~E115F00h	216.09	Negative	216.02783	1081	216.02798	C6H13NO5	Glucosamine	[M+K-2H]1-	-0.69
E115F00h~E129F00h	217.18	Negative	217.02960	848	-	-	37Cl Isotope of Glucose	[M+Cl]1-	-
E115F00h~E485F00h	218.09	Negative	217.97904	1438	-	-	Unknown	-	-
			218.10321	603	218.10340	C9H17NO5	Pantothenate	[M-H]1-	-0.86
2N=10~2N=30	220.09	Negative	219.97840	848	219.97833	C3H8NO6P	O-Phospho serine	[M+Cl]1-	0.33
2N=10~2N=20	221.09	Negative	220.94935	5273	220.94940	C7H4O6	Chelidonate	[M+K-2H]1-	-0.20
			221.06666	684	221.06668	C8H14O7	6-Acetyl glucose	[M-H]1-	-0.08
E137F00h~E485F00h	222.09	Negative	221.97526	502	-	-	37Cl Isotope of O-Phospho serine	[M+Cl]1-	-
			222.07707	653	222.07718	C11H13NO4	N-Acetyl tyrosine	[M-H]1-	-0.50
E114F00h~E137F00h	223.09	Negative	222.94148	1693	222.94155	C3H7O7P	3-Phospho glycerate	[M+K-2H]1-	-0.31
			223.06103	646	223.06120	C11H12O5	Sinapate	[M-H]1-	-0.75
			223.13403	688	223.13397	C13H20O3	Vomifoliol	[M-H]1-	0.28
E160F00h~E176F00h	224.09	Negative	224.05636	785	224.05672	C11H11N2O2	Tryptophan	[M+Na-2H]1-	-1.61
E114F00h~E480F00h	228.09	Negative	227.93053	1037	-	-	Unknown	-	-
			228.05118	403	228.05136	C9H11NO6	4,5-Seco-DOPA	[M-H]1-	-0.80
			228.06396	769	228.06421	C11H13NO3	N-Acetyl-phenylalanine	[M+Na-2H]1-	-1.10
2N=20~2N=30	229.09	Negative	228.97551	2757	228.97561	C6H8O7	Isocitrate	[M+K-2H]1-	-0.44
			228.99346	1296	-	-	Unknown	-	-
			229.01166	1801	229.01188	C5H11O8P	Ribose 1-phosphate	[M-H]1-	-0.97
E129F00h~E137F00h	231.09	Negative	231.03861	495	231.03873	C9H10N2O4	N-Carbamoyl-p-hydroxy-phenylglycine	[M+Na-2H]1-	-0.50
			231.08733	793	231.08741	C5H8O3	2-Oxo valerate	[2M-H]1-	-0.36
			231.09849	656	231.09865	C9H16N2O5	$\gamma$ -Glutamyl- $\gamma$ -aminobutyrate	[M-H]1-	-0.68
2N=20~2N=30	232.09	Negative	232.01155	480	232.01048	C4H10N3O5P	Phospho creatine	[M+Na-2H]1-	4.62
E114F00h~E480F00h	236.09	Negative	235.97342	509	235.97318	C4H10NO6P	O-Phospho homoserine	[M+K-2H]1-	1.01
			235.98144	1156	-	-	Unknown	-	-
E114F00h~E115F00h	237.09	Negative	236.95692	406	236.95720	C4H9O7P	Erythrose 4-phosphate	[M+K-2H]1-	-1.17
			237.00822	1151	-	-	Unknown	-	-

E114F00h~E333F00h	239.09	Negative	237.06160	1647	237.06159	C8H14O8	3-Deoxy-manno-octulosonate	[M-H]1-	0.03
			237.07694	555	237.07685	C12H14O5	3,4,5-Trimethoxy cinnamate	[M-H]1-	0.39
			239.05898	345	239.05928	C11H12N2O2	Tryptophan	[M+Cl]1-	-1.25
			239.07711	824	239.07724	C4H8O4	Threose	[2M-H]1-	-0.56
E162F00h~E485F00h	240.09	Negative	239.95843	1747	-	-	Unknown	-	-
			239.96779	855	239.96779	C7H7O7	4-Oxobut-1-ene-1,2,4-tricarboxylate	[M+K-2H]1-	0.02
			240.05124	586	240.05136	C10H11NO6	2,3-Dihydroxy benzoyl serine	[M-H]1-	-0.51
			240.97561	2333	240.97561	C7H8O7	Oxalo glutarate	[M+K-2H]1-	0.00
E129F00h~E160F00h	241.09	Negative	241.01201	2478	241.01206	C7H10O7	Homocitrate	[M+Cl]1-	-0.19
			244.93010	2022	-	-	Unknown	-	-
			245.04323	2492	245.04314	C11H12O5	Sinapate	[M+Na-2H]1-	0.36
			245.05428	625	245.05438	C10H12N2O4	3-Hydroxy kynurenine	[M+Na-2H]1-	-0.39
2N=20~2N=30	247.09	Negative	246.97783	495	246.97782	C2H5O4P	Phosphono acetaldehyde	[2M-H]1-	0.04
			246.99723	7379	246.99605	C3H8OS2	6-S-Acetyl-dihydro lipoate	[2M-H]1-	4.78
			247.01670	406	247.01669	C14H10O2	Phenanthrene-3,4-diol	[M+K-2H]1-	0.06
			251.05613	2649	251.05611	C6H6O3	Maltol	[2M-H]1-	0.07
2N=10~2N=30	251.18	Negative	252.95123	603	252.95211	C4H9O8P	3-Phospho erythronate	[M+K-2H]1-	-3.49
2N=10~2N=30	253.09	Negative	253.07180	939	253.07073	C6H15N4O5P	N-Phospho arginine	[M-H]1-	4.22
E162F00h~E260F00h	255.27	Negative	253.09292	519	253.09289	C9H18O8	Galactosyl glycerol	[M-H]1-	0.11
			253.21762	402	253.21730	C16H30O2	Hexadecenoate	[M-H]1-	1.25
			255.08754	1199	255.08741	C12H16O6	Phenyl galactoside	[M-H]1-	0.50
			255.12372	765	255.12273	C12H18N4O	Benzoyl agmatine	[M+Na-2H]1-	3.88
2N=10~2N=20	257.08	Negative	255.23299	2236	255.23295	C16H32O2	Palmitate	[M-H]1-	0.14
			257.00685	661	257.00680	C6H11O9P	Glucono-1,5-lactone 6-phosphate	[M-H]1-	0.21
			257.03031	745	257.02922	C9H8N4O4	Xanthopterin-B2	[M+Na-2H]1-	4.23
			259.02271	24036	259.02245	C6H13O9P	Glucose 6-phosphate	[M-H]1-	1.02
E114F00h~E176F00h	263.18	Negative	262.91203	1135	-	-	Unknown	-	-
			263.05588	901	263.05520	C9H14N4O3	Carnosine	[M+K-2H]1-	2.60
			263.15019	813	263.15001	C6H12O3	Leucate	[2M-H]1-	0.67
			264.95243	476	264.95200	C3H8O10P2	Glycerate 1,3-biphosphate	[M-H]1-	1.63
E114F00h~E480F00h	265.18	Negative	265.09293	844	265.09182	C9H16N4O4	Amidino proclavaminat	[M+Na-2H]1-	4.17
			265.14791	6072	265.14791	C12H26O4S	Sodium lauryl sulfate	[M-H]1-	0.01
			265.98249	824	265.98375	C5H12NO7P	5-Phospho ribosyl amine	[M+K-2H]1-	-4.73

E260F00h~E484F00h	267.18	Negative	265.99195	1102	-	-	Unknown	-	-
			266.01790	1459	-	-	Unknown	-	-
			267.01461	1599	-	-	Unknown	-	-
			267.02483	784	267.02513	C6H15O8P	2-Deoxyglucose-6-phosphate	[M+Na-2H]1-	-1.11
E137F00h~E485F00h	269.18	Negative	267.07180	1227	267.07216	C9H16O9	Glucosyl glycerate	[M-H]1-	-1.34
			268.94684	2465	-	-	Unknown	-	-
			269.03362	1109	269.03340	C12H12N2O3	Pheno barbital	[M+K-2H]1-	0.83
			269.24830	549	269.24860	C17H34O2	Methyl palmitate	[M-H]1-	-1.13
2N=20~2N=30	271	Negative	271.00938	1009	-	-	Unknown	-	-
2N=10~2N=30	274.08	Negative	271.02239	904	271.02256	C9H14O7	(Homo)3-citrate	[M+K-2H]1-	-0.63
			273.95757	1335	-	-	Unknown	-	-
			274.03306	464	274.03346	C8H15NO7	N-Acetyl glucosamine	[M+K-2H]1-	-1.46
			277.21765	1726	277.21730	C18H30O2	Linolenate	[M-H]1-	1.25
E114F00h~E137F00h	278.18	Negative	278.06978	1215	278.07018	C13H13N3O2	Methoxy-PU	[M+Cl]1-	-1.43
2N=10~2N=20	279.18	Negative	279.23263	494	279.23295	C18H32O2	Linoleate	[M-H]1-	-1.16
2N=10~2N=20	281.18	Negative	280.98477	1091	280.98341	C6H13O8P	Fucose 1-phosphate	[M+K-2H]1-	4.83
			280.99473	1099	280.99488	C6H12O9S	Glucose 6-sulfate	[M+Na-2H]1-	-0.52
			281.06677	26489	281.06668	C26H28O14	Apigenin 7-O-[β-apiosyl-(1→2)-β-glucoside]	[M-2H]2-	0.33
			281.08733	595	281.08781	C10H18O9	Xylobiose	[M-H]1-	-1.70
2N=20~2N=30	289.18	Negative	289.03300	5583	289.03301	C7H15O10P	Sedoheptulose 7-phosphate	[M-H]1-	-0.04
			289.11525	869	289.11536	C10H18N4O6	Arginino succinate	[M-H]1-	-0.38
			293.02216	741	293.02217	C15H12O4	3',5'-Dihydroxy flavanone	[M+K-2H]1-	-0.02
			293.06675	1960	-	-	Unknown	-	-
E114F00h~E480F00h	295.18	Negative	295.10315	1023	295.10239	C10H18N4O5	N2-Succinyl arginine	[M+Na-2H]1-	2.58
E114F00h~E137F00h	297.18	Negative	297.03549	8420	-	-	Unknown	-	-
			297.06165	10568	297.06159	C13H14O8	Benzoyl glucuronide	[M-H]1-	0.19
			297.15281	5740	-	-	Unknown	-	-
			298.03371	885	298.03346	C10H15NO7	Hymexazol N-glucoside	[M+K-2H]1-	0.84
E137F00h~E160F00h	303.18	Negative	298.15616	1012	-	-	Unknown	-	-
			302.90814	908	302.90788	C3H8O10P2	Glycerate 1,3-biphosphate	[M+K-2H]1-	0.86
			303.06125	997	303.06134	C10H14N6O3	3'-Amino-3'-deoxyadenosine	[M+K-2H]1-	-0.31
			308.97803	15770	308.97821	C5H12O11P2	Ribose-1,5-bisphosphate	[M-H]1-	-0.60
E129F00h~E333F00h	309.18	Negative	309.11851	1023	-	-	Unknown	-	-

E129F00h~E137F00h	311.18	Negative	311.07690	9334	311.07724	C14H16O8	O-Feruloyl threonate	[M-H]1-	-1.10
			311.11357	1030	311.11363	C15H20O7	4-Hydroxycinnamyl alcohol 4-glucoside	[M-H]1-	-0.19
			311.16878	21569	-	-	Unknown	-	-
2N=10~2N=30	312.18	Negative	312.08030	1205	-	-	Unknown	-	-
			312.17183	2433	-	-	13C Isotope of 311.16878	-	-
			315.07183	19901	315.07243	C14H16NO6	Indican	[M+Na-2H]1-	-1.91
2N=10~2N=20	315.18	Negative	315.10845	749	315.10854	C7H10O4	2-Isopropyl maleate	[2M-H]1-	-0.30
			316.07486	1936	-	-	13C Isotope of Indican	[M+Na-2H]1-	-
			316.10376	430	316.10272	C12H17N5O4	N6,N6-Dimethyl adenosine	[M+Na-2H]1-	3.28
2N=10~2N=30	316.18	Negative	319.02254	721	319.02241	C16H10O6	2',7-Dihydroxy-4',5'-methylene dioxysiflavone	[M+Na-2H]1-	0.41
			319.04461	794	319.04594	C15H12O8	2,3-Dihydro quercetagenin	[M-H]1-	-4.18
			319.09060	825	319.08954	C5H8N2O4	Formimino aspartate	[2M-H]1-	3.33
2N=10~2N=20	319.18	Negative	319.13943	638	319.13984	C14H24O8	Octanoyl glucuronide	[M-H]1-	-1.29
			323.02805	1327	323.02859	C9H13N2O9P	Uridine monophosphate	[M-H]1-	-1.68
			323.07756	1173	-	-	Unknown	-	-
E114F00h~E160F00h	323.18	Negative	323.13409	2537	323.13563	C7H14O2S	7-Mercapto heptanoate	[2M-H]1-	-4.77
			326.18760	2583	326.18917	C19H31NO	17 $\alpha$ -Azahomoandrost-5-en-3 $\beta$ -ol	[M+K-2H]1-	-4.81
			326.95252	937	326.95251	C6H11O11P	3-Phospho glucarate	[M+K-2H]1-	0.04
E115F00h~E129F00h	326.18	Negative	326.98835	786	326.98889	C7H15O10P	Sedoheptulose-7-phosphate	[M+K-2H]1-	-1.66
			327.05765	743	327.05690	C5H8O6	2-Dehydro xylonate	[2M-H]1-	2.28
			327.07158	660	327.07124	C10H18N4O6	Arginino succinate	[M+K-2H]1-	1.04
E260F00h~E484F00h	327.18	Negative	327.08849	688	327.08741	C9H8O3	p-Coumarate	[2M-H]1-	3.29
			327.21752	1026	327.21770	C18H32O5	9,10,11-Trihydroxy-12,15-octadecadienoate	[M-H]1-	-0.54
			329.01507	667	329.01562	C9H13N2O8P	dUMP	[M+Na-2H]1-	-1.68
E129F00h~E137F00h	329.18	Negative	329.06641	1020	329.06668	C17H14O7	3',4',5-Trihydroxy-3,7-dimethoxyflavone	[M-H]1-	-0.81
			329.23337	686	329.23335	C18H34O5	9,12,13-Trihydroxy-10-octadecenoate	[M-H]1-	0.07
			337.09263	1224	337.09289	C16H18O8	p-Coumaroyl quinate	[M-H]1-	-0.78
E333F00h~E480F00h	337.09	Negative	339.82072	769	-	-	37Cl Isotope of 337.82329	-	-
E114F00h~E115F00h	340.09	Negative	340.20246	1783	-	-	Unknown	-	-
2N=20~2N=30	341.27	Negative	341.10886	210747	341.10894	C12H22O11	Sucrose	[M-H]1-	-0.23
2N=20~2N=30	342.18	Negative	342.11240	9428	-	-	13C Isotope of Sucrose	[M-H]1-	-
E114F00h~E115F00h	343.18	Negative	343.11396	1357	-	-	Unknown	-	-
			343.16837	431	343.16810	C19H30O3	5-Androstene-3 $\beta$ ,16 $\alpha$ ,17 $\alpha$ -triol	[M+K-2H]1-	0.79

2N=10~2N=20	344.09	Negative	344.09872	713	344.09913	C12H23N2O7	Fructose lysine	[M+K-2H]1-	-1.20
			344.11702	970	-	-	Unknown	-	-
2N=10~2N=20	346.09	Negative	346.05500	3522	346.05581	C10H14N5O7P	Adenosine monophosphate	[M-H]1-	-2.34
			346.09947	1006	-	-	Unknown	-	-
2N=10~2N=30	347.09	Negative	346.93344	1097	346.93410	C5H12O11P2	Ribose 1,5-bisphosphate	[M+K-2H]1-	-1.89
			347.02528	821	347.02560	C6H6O6	Dehydro ascorbate	[2M-H]1-	-0.93
			347.07521	646	347.07484	C15H18O8	<i>p</i> -Coumaroyl glucose	[M+Na-2H]1-	1.07
			347.17068	763	347.17114	C8H14O4	Suberate	[2M-H]1-	-1.33
E137F00h~E480F00h	349.09	Negative	349.03274	584	349.03198	C6H16N4O9P2	N-Phospho lombricine	[M-H]1-	2.18
			349.15070	1518	-	-	Unknown	-	-
E129F00h~E160F00h	355.18	Negative	355.10285	808	355.10346	C16H20O9	1- <i>O</i> -Feruloyl glucose	[M-H]1-	-1.71
E114F00h~E137F00h	359.18	Negative	359.11957	604	359.11950	C6H12O6	Glucose	[2M-H]1-	0.19
E115F00h~E137F00h	362.18	Negative	362.04972	960	362.05073	C10H14N5O8P	Guanosine monophosphate	[M-H]1-	-2.78
2N=10~2N=30	364.18	Negative	364.06671	826	364.06500	C14H17NO9	DIBOA-glucoside	[M+Na-2H]1-	4.69
			364.11587	635	364.11664	C19H21NO5	Sinapoyl tyramine	[M+Na-2H]1-	-2.12
2N=10~2N=30	367.18	Negative	367.10250	3571	367.10346	C17H20O9	<i>O</i> -Feruloyl quinate	[M-H]1-	-2.61
2N=10~2N=20	369.09	Negative	368.99910	1099	368.99934	C7H16O13P2	Sedoheptulose-1,7-bisphosphate	[M-H]1-	-0.66
E114F00h~E129F00h	371.27	Negative	371.09729	7226	371.09837	C8H10O5	2-Hydroxy-3-Ccarboxy-4,5-cyclopropylhex-5-enoate	[2M-H]1-	-2.92
			371.13456	650	371.13476	C17H24O9	Syringin	[M-H]1-	-0.53
			371.16320	907	371.16302	C20H30O4	PGA2	[M+K-2H]1-	0.50
2N=10~2N=20	377.27	Negative	377.08556	36887	377.08562	C12H22O11	Sucrose	[M+Cl]1-	-0.15
2N=10~2N=20	379.18	Negative	379.04405	1081	379.04369	C15H18O9	1-Caffeoyl- $\beta$ -glucose	[M+K-2H]1-	0.95
			379.08156	8368	-	-	<sup>37</sup> Cl Isotope of Sucrose	[M+Cl]1-	-
			379.15964	2819	379.16097	C8H14O5	3-Hydroxy suberate	[2M-H]1-	-3.52
2N=10~2N=20	380.18	Negative	380.04265	1257	380.04430	C20H13N3O3	Violacein	[M+K-2H]1-	-4.33
2N=10~2N=30	381.18	Negative	381.04777	737	381.04847	C10H15N6O6P	2-Amino-AMP	[M+Cl]1-	-1.84
			381.08684	552	-	-	Unknown	-	-
			381.13136	592	381.13196	C20H24O6	Lactol	[M+Na-2H]1-	-1.57
2N=20~2N=30	382.18	Negative	382.01666	3079	-	-	Unknown	-	-
			382.10196	652	382.10044	C14H17N5O8	Succinyl adenosine	[M-H]1-	3.98
			382.13436	680	382.13549	C14H25NO11	Galactosyl-3-N-acetyl- $\beta$ -galactosamine	[M-H]1-	-2.95
2N=10~2N=20	385.18	Negative	385.11286	2095	385.11402	C17H22O10	1- <i>O</i> -sinapoyl- $\beta$ -D-glucose	[M-H]1-	-3.02
2N=10~2N=20	387.18	Negative	387.11385	42068	-	-	Unknown	-	-

2N=20~2N=30	388.18	Negative	387.13057	740	387.12967	C17H24O10	Verbenalin	[M-H]1-	2.32
			387.16508	881	387.16606	C18H28O9	Tuberonate glucoside	[M-H]1-	-2.53
			388.11613	4081	388.11679	C18H25NO6	Anacrotine	[M+K-2H]1-	-1.71
2N=20~2N=30	389.18	Negative	388.11943	2987	-	-	Unknown	-	-
			389.10703	524	389.10894	C16H22O11	Monotropein	[M-H]1-	-4.90
			389.11984	790	-	-	13C Isotope of Anacrotine	[M+K-2H]1-	-
E137F00h~E162F00h	393.09	Negative	389.14476	567	389.14532	C17H26O10	Loganin	[M-H]1-	-1.45
			393.04432	838	393.04393	C15H16O11	2-O-Caffeoyl glucarate	[M+Na-2H]1-	0.98
			393.17526	804	393.17555	C16H28N4O6	(Ac)2-Lys-Ala-Ala	[M+Na-2H]1-	-0.75
E115F00h~E485F00h	397.09	Negative	397.03990	932	397.04188	C4H10NO6P	Phospho threonine	[2M-H]1-	-4.98
			397.07609	2600	-	-	Unknown	-	-
			397.07960	2192	397.08074	C18H20N2O6	Di tyrosine	[M+K-2H]1-	-2.88
E114F00h~E115F00h	398.18	Negative	397.11394	829	397.11295	C17H20N4O6	Riboflavin	[M+Na-2H]1-	2.48
			398.02072	821	398.01972	C10H19NO10S2	3-Hydroxypropyl glucosinolate	[M+Na-2H]1-	2.53
			398.08047	819	-	-	Unknown	-	-
E115F00h~E333F00h	399.18	Negative	398.13102	727	-	-	Unknown	-	-
			399.01865	1071	-	-	Unknown	-	-
			399.10657	672	399.10854	C21H20O8	Flavonol 3-O-galactoside	[M-H]1-	-4.94
2N=10~2N=20	407.18	Negative	399.13059	832	399.12860	C17H22N4O6	Reduced Riboflavin	[M+Na-2H]1-	4.98
			407.06487	1374	-	-	Unknown	-	-
			407.15687	739	407.15676	C9H16O3S	2-Oxo-8-methyl thiooctanoate	[2M-H]1-	0.27
2N=10~2N=30	408.09	Negative	407.19153	913	407.19227	C9H16O5	Diethyl-2-methyl-3-hydroxy succinate	[2M-H]1-	-1.82
			407.78586	835	-	-	Unknown	-	-
			408.14996	688	408.15114	C16H27NO11	Linustatin	[M-H]1-	-2.88
E114F00h~E137F00h	413.18	Negative	413.08269	1090	-	-	Unknown	-	-
			413.14330	3219	413.14305	C8H17NOS2	Dihydro lipoamide	[2M-H]1-	0.61
			413.21669	530	-	-	Unknown	-	-
E480F00h~E485F00h	419.09	Negative	419.09701	1150	419.09837	C10H10O5	5-Hydroxy ferulate	[2M-H]1-	-3.25
			419.11131	748	419.11191	C15H22N6O5S	S-Adenosyl methionine	[M+Na-2H]1-	-1.43
			419.15588	717	-	-	Unknown	-	-
E333F00h~E480F00h	420.18	Negative	419.19186	833	-	-	Unknown	-	-
			420.09271	936	420.09137	C14H25NO11	$\beta$ -1,4-Mannose-N-acetyl glucosamine	[M+K-2H]1-	3.19
			421.07450	959	421.07527	C12H23O14P	Sucrose 6-phosphate	[M-H]1-	-1.83

2N=20~2N=30	423.18	Negative	421.15300	797	-	-	Unknown	-	-
			423.15902	755	423.16018	C14H12O2	Phenanthrene-9,10-dihydrodiol	[2M-H]1-	-2.75
			423.16439	721	-	-	Unknown	-	-
2N=20~2N=30	425.18	Negative	423.17750	569	-	-	Unknown	-	-
			425.06861	872	-	-	Unknown	-	-
			425.08145	634	425.08064	C13H22N4O8S2	S-Glutathionyl cysteine	[M-H]1-	1.91
E114F00h~E480F00h	427.18	Negative	425.18283	366	425.18171	C21H30O9	Abscisate glucose ester	[M-H]1-	2.64
			427.02514	981	-	-	Unknown	-	-
			427.15953	827	427.16097	C20H28O10	Furcatin	[M-H]1-	-3.38
E176F00h~E484F00h	429.18	Negative	429.01887	1017	429.01698	C14H18NO9S2	Benzyl glucosinolate	[M+Na-2H]1-	4.42
E160F00h~E485F00h	431.18	Negative	429.10258	1004	429.10448	C5H14NO6P	1-Glycero phosphoryl ethanolamine	[2M-H]1-	-4.42
			431.09630	3091	431.09837	C21H20O10	Apigenin 7-O-β-Glucoside	[M-H]1-	-4.81
			431.10226	3774	-	-	Unknown	-	-
2N=10~2N=20	435.18	Negative	431.21946	657	431.21801	C19H39O7P	PA(16:0/0:0)[U]	[M+Na-2H]1-	3.36
			434.88340	1038	-	-	Unknown	-	-
			435.09079	2048	435.09088	C18H22O11	Asperuloside	[M+Na-2H]1-	-0.21
2N=10~2N=20	437.18	Negative	435.22173	654	435.22357	C10H18O5	3-Hydroxy sebacate	[2M-H]1-	-4.23
			437.07430	1854	437.07451	C6H10N3O4P	Hydroxymethyl pyrimidine phosphate	[2M-H]1-	-0.49
			437.16579	680	-	-	Unknown	-	-
E115F00h~E129F00h	440.18	Negative	437.20118	733	-	-	Unknown	-	-
			440.07767	2101	-	-	Unknown	-	-
			440.08685	4486	-	-	Unknown	-	-
E114F00h~E480F00h	443.18	Negative	443.11784	941	-	-	Unknown	-	-
			443.15395	769	443.15588	C19H22N7O6	7,8-Dihydrofolate	[M-H]1-	-4.36
			443.19239	595	-	-	Unknown	-	-
2N=10~2N=20	447.18	Negative	447.09066	5773	-	-	Unknown	-	-
			447.11578	661	-	-	Unknown	-	-
			447.13322	1139	-	-	Unknown	-	-
E114F00h~E480F00h	449.09	Negative	447.15353	954	447.15214	C10H12N2O4	3-Hydroxy kynurenine	[2M-H]1-	3.11
			449.10829	750	449.10894	C21H22O11	Pentahydroxychalcone 4'-O-glucoside	[M-H]1-	-1.44
			453.07977	983	453.08032	C21H20O10	Apigenin 7-O-β-Glucoside	[M+Na-2H]1-	-1.21
E129F00h~E137F00h	453.18	Negative	453.10262	1018	-	-	Unknown	-	-
			453.15216	339	453.15147	C10H13NO5	Aroenate	[2M-H]1-	1.52

2N=20~2N=30	455.18	Negative	455.09684	968	455.09734	C17H21N4O9P	Flavin Mononucleotide	[M-H]1-	-1.10
			455.11768	466	-	-	Unknown	-	-
			455.19400	497	-	-	Unknown	-	-
2N=10~2N=30	459.09	Negative	459.14936	733	459.15080	C20H28O12	Paeonolide	[M-H]1-	-3.14
E129F00h~E160F00h	463.18	Negative	463.04259	1168	-	-	Unknown	-	-
			463.21764	700	463.21936	C11H20O3S	2-Oxo-10-Methylthiodecanoate	[2M-H]1-	-3.72
2N=10~2N=30	469.18	Negative	469.07361	731	469.07523	C21H20O11	Kaempferol 7-O-Glucoside	[M+Na-2H]1-	-3.46
Pilar analysis	471.18	Negative	471.07287	1714	-	-	Unknown	-	-
			471.09209	639	471.09088	C21H22O11	Pentahydroxychalcone 4'-O-Glucoside	[M+Na-2H]1-	2.56
			471.07287	1714	-	-	Unknown	-	-
2N=20~2N=30	475.09	Negative	475.12810	1774	475.13046	C8H14O8	3-Deoxy-manno-octulosonate	[2M-H]1-	-4.97
			475.14890	1095	-	-	Unknown	-	-
			475.21772	641	475.21608	C20H38O11	Octyl β-1,6-galactofuranosyl-α-glucopyranoside	[M+Na-2H]1-	3.44
E115F00h~E485F00h	479.18	Negative	479.03946	585	479.04044	C6H12N2O4S2	Cystine	[2M-H]1-	-2.04
			479.10104	664	479.09971	C13H8N2O3	2-Hydroxyphenazine-1-carboxylate	[2M-H]1-	2.78
E114F00h~E137F00h	481.27	Negative	481.25439	1224	481.25720	C22H43O9P	2-16:1-LysoPG	[M-H]1-	-5.83
2N=10~2N=30	483.27	Negative	483.27087	1074	483.27285	C22H45O9P	PG(16:0/0:0)	[M-H]1-	-4.09
2N=10~2N=30	487.18	Negative	487.15628	647	487.15592	C14H29N6O11P	O-1,4-α-Dihydrostreptosyl-streptidine 6-phosphate	[M-H]1-	0.74
			487.17619	2177	487.17516	C28H26N4O3	Staurosporine	[M+Na-2H]1-	2.12
2N=10~2N=20	488.18	Negative	488.06261	2308	-	-	Unknown	-	-
			488.10807	663	488.10957	C18H23N6O7S	(4-Azidophenacyl)glutathione	[M+Na-2H]1-	-3.06
			488.15921	3655	-	-	Unknown	-	-
			488.18137	1209	-	-	Unknown	-	-
E114F00h~E484F00h	493.18	Negative	493.12949	565	493.13113	C9H13NO7	N-Succinyl glutamate	[2M-H]1-	-3.33
E114F00h~E480F00h	503.27	Negative	503.15904	1878	503.16176	C18H32O16	Raffinose	[M-H]1-	-5.41
2N=10~2N=20	510	Negative	510.10117	545	510.10193	C20H27NO12	Proteacin	[M+K-2H]1-	-1.50
			510.21737	537	510.21672	C25H37NO8S	ω-carboxy-N-acetyl-Lte4	[M-H]1-	1.28
2N=10~2N=30	513.17	Negative	513.10357	830	513.10145	C23H24O12	Cicerin-7-O-glucoside	[M+Na-2H]1-	4.14
2N=20~2N=30	515.17	Negative	515.10021	1036	515.09837	C14H10O5	Gentisin	[2M-H]1-	3.57
			515.12404	1005	-	-	Unknown	-	-
E115F00h~E176F00h	521	Negative	521.33212	758	521.33312	C26H50O10	Tween 20	[M-H]1-	-1.92
E115F00h~E485F00h	534.09	Negative	534.17408	1007	534.17455	C27H31NO9	13-Deoxydaunorubicin	[M+Na-2H]1-	-0.88
E333F00h~E480F00h	535.27	Negative	535.03503	1020	535.03719	C14H22N2O16P2	UDP-Arabinose	[M-H]1-	-4.03



E114F00h~E137F00h	539.27	Negative	539.13550	2797	539.13844	C18H32O16	Raffinose	[M+Cl]1-	-5.45
<b>E114F00h~E137F00h</b>	<b>540.17</b>	<b>Negative</b>	<b>540.03362</b>	<b>1018</b>	-	-	<b>Unknown</b>	-	-
			<b>540.05642</b>	<b>1055</b>	<b>540.05384</b>	<b>C15H21N5O13P2</b>	<b>Cyclic ADP-ribose</b>	<b>[M-H]1-</b>	<b>4.78</b>
E137F00h~E160F00h	541.27	Negative	541.13280	1033	-	-	37Cl Isotope of raffinose	[M+Cl]1-	-
2N=10~2N=20	563.27	Negative	563.13800	6060	563.14063	C26H28O14	Apigenin 7-O-[β-apiosyl-(1->2)-β-glucoside]	[M-H]1-	-4.68
2N=10~2N=20	564.27	Negative	564.14164	1829	-	-	13C Isotope of Apigenin 7-O-[β-apiosyl-(1->2)-β-glucoside]	[M-H]1-	-
<b>E480F00h~E485F00h</b>	<b>565.17</b>	<b>Negative</b>	<b>565.04434</b>	<b>2039</b>	-	-	<b>Unknown</b>	-	-
			<b>565.14542</b>	<b>770</b>	<b>565.14816</b>	<b>C28H32O10</b>	<b>1,2-Bis-O-sinapoyl-β-glucoside</b>	<b>[M+K-2H]1-</b>	<b>-4.84</b>
<b>E114F00h~E137F00h</b>	<b>566.27</b>	<b>Negative</b>	<b>566.04925</b>	<b>1087</b>	-	-	<b>Unknown</b>	-	-
			<b>566.15362</b>	<b>3011</b>	<b>566.15604</b>	<b>C27H31O11</b>	<b>Curcumin monoglucoside</b>	<b>[M+Cl]1-</b>	<b>-4.28</b>
E137F00h~E162F00h	567.17	Negative	567.15793	810	-	-	13C Isotope of Curcumin monoglucoside	[M+Cl]1-	-
2N=10~2N=30	<b>588.09</b>	<b>Negative</b>	<b>588.07437</b>	<b>566</b>	<b>588.07497</b>	<b>C16H25N5O15P2</b>	<b>ADP-Glucose</b>	<b>[M-H]1-</b>	<b>-1.02</b>
			<b>588.13461</b>	<b>934</b>	<b>588.13651</b>	<b>C19H33N3O14P2</b>	<b>dTDP-4-dimethylamino-4,6-dideoxy-5-C-methyl-mannose</b>	<b>[M-H]1-</b>	<b>-3.22</b>
<b>E114F00h~E137F00h</b>	<b>593.27</b>	<b>Negative</b>	<b>593.15028</b>	<b>1107</b>	<b>593.15120</b>	<b>C27H30O15</b>	<b>Isoorientin 2''-O-rhamnoside</b>	<b>[M-H]1-</b>	<b>-1.55</b>
2N=20~2N=30	<b>597.17</b>	<b>Negative</b>	<b>597.21676</b>	<b>708</b>	<b>597.21951</b>	<b>C10H22NO7P</b>	<b>PC(0:0/2:0)</b>	<b>[2M-H]1-</b>	<b>-4.60</b>
2N=20~2N=30	<b>601.17</b>	<b>Negative</b>	<b>601.13443</b>	<b>1974</b>	<b>601.13290</b>	<b>C27H32O13</b>	<b>Pinocembrin 7-rhamnosyl glucoside</b>	<b>[M+K-2H]1-</b>	<b>2.55</b>
2N=10~2N=20	<b>623.17</b>	<b>Negative</b>	<b>623.18176</b>	<b>1037</b>	<b>623.18316</b>	<b>C25H30N8O10</b>	<b>10-Formyl tetrahydrofolyl Glutamate</b>	<b>[M+Na-2H]1-</b>	<b>-2.25</b>
Pilar analysis	<b>637.09</b>	<b>Negative</b>	<b>637.17555</b>	<b>497</b>	<b>637.17741</b>	<b>C29H34O16</b>	<b>Dalpatein 7-O-β-D-apiofuranosyl-(1-&gt;6)-β-D-glucopyranoside</b>	<b>[M-H]1-</b>	<b>-2.92</b>
2N=20~2N=30	<b>640.9</b>	<b>Negative</b>	<b>641.12809</b>	<b>1069</b>	-	-	<b>Unknown</b>	-	-
			<b>641.20676</b>	<b>493</b>	<b>641.20644</b>	<b>C13H23NO4S2</b>	<b>Glutaryl dihydro lipamide</b>	<b>[2M-H]1-</b>	<b>0.50</b>
E129F00h~E176F00h	683.27	Negative	683.22272	1119	683.22515	C12H22O11	Sucrose	[2M-H]1-	-3.56
2N=10~2N=20	711.45	Negative	711.21064	847	711.21419	C16H20O9	1-O-Feruloyl-β-glucose	[2M-H]1-	-5.00
2N=10~2N=20	<b>719.63</b>	<b>Negative</b>	<b>719.48650</b>	<b>618</b>	<b>719.48686</b>	<b>C38H73O10P</b>	<b>PG(16:0/16:1(9Z))</b>	<b>[M-H]1-</b>	<b>-0.50</b>
E115F00h~E129F00h	741.63	Negative	741.46610	2498	741.46881	C38H73O10P	PG(16:0/16:1(9Z))	[M+Na-2H]1-	-3.65
<b>E137F00h~E480F00h</b>	<b>743.63</b>	<b>Negative</b>	<b>743.48374</b>	<b>844</b>	<b>743.48686</b>	<b>C40H73O10P</b>	<b>PG(16:0/18:3(6Z,9Z,12Z))</b>	<b>[M-H]1-</b>	<b>-4.20</b>
			<b>743.79062</b>	<b>700</b>	-	-	<b>Unknown</b>	-	-
2N=10~2N=30	<b>745.63</b>	<b>Negative</b>	<b>745.50062</b>	<b>438</b>	<b>745.50251</b>	<b>C40H75O10P</b>	<b>PG(18:1(11Z)/16:1(9Z))</b>	<b>[M-H]1-</b>	<b>-2.54</b>
<b>E115F00h~E129F00h</b>	<b>773.36</b>	<b>Negative</b>	<b>773.26046</b>	<b>852</b>	-	-	<b>Unknown</b>	-	-
			<b>773.52328</b>	<b>363</b>	<b>773.52092</b>	<b>C45H74O10</b>	<b>1,2 Di-(9Z,12Z,15Z-Octadecatrienoyl)-3-O-β-galactosyl-sn-glycerol</b>	<b>[M-H]1-</b>	<b>3.05</b>
<b>E115F00h~E137F00h</b>	<b>793.72</b>	<b>Negative</b>	<b>793.51235</b>	<b>340</b>	<b>793.51413</b>	<b>C41H78O12S</b>	<b>1,2-Dihexadecanoyl-3-(6'-sulfo-α-D-quinovosyl)-sn-glycerol</b>	<b>[M-H]1-</b>	<b>-2.24</b>
2N=10~2N=20	<b>815.72</b>	<b>Negative</b>	<b>815.49378</b>	<b>1974</b>	<b>815.49510</b>	<b>C23H36O6</b>	<b>15R-PGE2 methyl ester, 15-acetate</b>	<b>[2M-H]1-</b>	<b>-1.62</b>
2N=10~2N=30	<b>819.63</b>	<b>Negative</b>	<b>819.52028</b>	<b>683</b>	<b>819.51816</b>	<b>C46H77O10P</b>	<b>PG(18:1(11Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))</b>	<b>[M-H]1-</b>	<b>2.59</b>
2N=10~2N=20	<b>831.63</b>	<b>Negative</b>	<b>831.50131</b>	<b>1049</b>	<b>831.50291</b>	<b>C43H77O13P</b>	<b>1-16:0-2-18:3-Phosphatidyl inositol</b>	<b>[M-H]1-</b>	<b>-1.92</b>

2N=10~2N=20	837.63	Negative	837.51583	1378	837.51343	C47H76O11	1-(9Z-Octadecenoyl)-2-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-3-O- $\alpha$ -glucuronyl-sn-glycerol	[M+Na-2H]1-	2.86
E129F00h~E176F00h	58.09	Positive	58.06525	105784	58.06513	C4H9NO2	$\gamma$ -Amino butyrate	[M+H-FA]1+	2.07
E115F00h~E137F00h	62.09	Positive	62.02391	573	62.02366	CH3NO2	Carbamate	[M+H]1+	4.11
			62.06016	8197	62.06004	C2H7NO	Ethanol amine	[M+H]1+	1.84
E176F00h~E485F00h	74.09	Positive	74.02360	2574	74.02366	C2H3NO2	Imino glycine	[M+H]1+	-0.77
			74.06000	11367	74.06004	C3H7NO	3-Amino propanal	[M+H]1+	-0.53
			74.09637	22483	74.09643	C4H11N	Butyl amine	[M+H]1+	-0.74
E137F00h~E162F00h	84.09	Positive	84.03311	5931	-	-	Unknown	-	-
			84.08072	58124	84.08078	C5H9N	Piperidine	[M+H]1+	-0.63
Pilar analysis	98	Positive	98.05794	1559	98.05764	C3H9NO	1-Amino propan-2-ol	[M+Na]1+	3.14
E115F00h~E480F00h	102	Positive	102.03358	117144	-	-	Unknown	-	-
			102.05467	21923	102.05496	C4H7NO2	Aceto acetamide	[M+H]1+	-2.80
			102.05637	23625	-	-	Unknown	-	-
			102.09102	2493	102.09134	C5H11NO	5-Amino pentanal	[M+H]1+	-3.14
E114F00h~E480F00h	106	Positive	106.04956	63524	106.04987	C3H7NO3	Serine	[M+H]1+	2.46
			106.05803	6506	-	-	Unknown	-	-
E115F00h~E260F00h	107	Positive	107.04871	1150	107.04914	C7H6O	Benzaldehyde	[M+H]1+	-4.04
			107.05285	1342	-	-	13C Isotope of Serine	[M+H]1+	-
			107.05749	4891	-	-	Unknown	-	-
2N=20~2N=30	111.54	Positive	111.55769	16203	111.55786	C7H15N3O5	1-Guanidino-1-deoxy-scyllo-inositol	[M+2H]2+	-1.55
E115F00h~E176F00h	113.45	Positive	113.54643	6717	-	-	Unknown	-	-
			113.55113	608	113.55104	C10H13N2O4	Porphobilinogen	[M+2H]2+	0.77
2N=10~2N=20	114	Positive	114.01677	8539	-	-	Unknown	-	-
			114.03965	53325	-	-	Unknown	-	-
			114.05459	1861	114.05496	C5H7NO2	Pyrroline 5-carboxylate	[M+H]1+	-3.21
			114.06571	1627	114.06619	C4H7N3O	Creatinine	[M+H]1+	-4.20
E114F00h~E176F00h	116	Positive	116.03384	2969	116.03422	C4H5NO3	Maleamate	[M+H]1+	-3.28
			116.03719	4024	-	-	41K Isotope of 114.03965	-	-
			116.07030	9205	116.07061	C5H9NO2	Proline	[M+H]1+	-2.64
2N=20~2N=30	118.09	Positive	118.08587	1468640	118.08626	C5H11NO2	Valine and Betaine	[M+H]1+	-3.27
2N=20~2N=30	119.09	Positive	119.06990	1033	119.07027	C5H10O3	2-Hydroxy valerate	[M+H]1+	-3.12
			119.08914	61037	-	-	13C Isotope of Valine and 13C Isotope of Betaine	[M+H]1+	-

E115F00h~E260F00h 2N=20~2N=30	120.09 123	Positive	120.06515	37635	120.06552	C4H9NO3	Threonine	[M+H] <sup>1+</sup>	-3.09
			122.92412	91633	-	-	Unknown	-	-
			123.03989	16616	123.04004	CH3NO2	Carbamate	[2M+H] <sup>1+</sup>	-1.18
			123.05488	1942	123.05529	C6H6N2O	Nicotinamide	[M+H] <sup>1+</sup>	-3.33
			123.06563	1708	123.06519	C4H10O4	Erythritol	[M+H] <sup>1+</sup>	3.60
E114F00h~E137F00h	123.9	Positive	123.07052	14008	-	-	Unknown	-	-
			124.06019	1635	124.06044	C3H6O4	Glycerate	[M+NH4] <sup>1+</sup>	-1.98
			124.07274	1141	124.07329	C5H11NO	4-Methyl aminobutanal	[M+Na] <sup>1+</sup>	-4.39
			124.07524	949	124.07569	C7H9NO	2-Amino- <i>m</i> -cresol	[M+H] <sup>1+</sup>	-3.63
			126.99838	1561	126.99783	C4H8S	Tetrahydro thiophene	[M+K] <sup>1+</sup>	4.31
E129F00h~E162F00h	127.09	Positive	127.03856	1568	127.03897	C6H6O3	1,2,3-Trihydroxy benzene	[M+H] <sup>1+</sup>	-3.24
			127.05142	1408	127.05197	C5H12O	2-Methyl butanol	[M+K] <sup>1+</sup>	-4.36
			127.12251	69695	127.12298	C7H14N2	1,5-Diazabicyclo nonane	[M+H] <sup>1+</sup>	-3.66
			128.01035	1790	128.01084	C3H7NO2	Alanine	[M+K] <sup>1+</sup>	-3.81
			128.03128	1312	128.03182	C3H7NO3	Serine	[M+Na] <sup>1+</sup>	-4.18
E137F00h~E162F00h	128	Positive	128.05521	10759	-	-	Unknown	-	-
			128.12580	2467	-	-	<sup>13</sup> C Isotope of 1,5-Diazabicyclo nonane	[M+H] <sup>1+</sup>	-
			132.06510	1328	132.06552	C5H9NO3	3-Hydroxy proline	[M+H] <sup>1+</sup>	-3.19
			132.10146	21097	132.10191	C6H13NO2	Leucine	[M+H] <sup>1+</sup>	-3.38
			132.54472	2360	132.54456	C9H17NO5	Pantothenate	[M+2Na] <sup>2+</sup>	1.22
E115F00h~E176F00h 2N=20~2N=30 2N=20~2N=30	132.44 138.09	Positive	138.03123	1982	138.03157	C5H9NO	N-Methyl-2-pyrrolidinone	[M+K] <sup>1+</sup>	-2.48
			138.05268	995	138.05255	C5H9NO2	Proline	[M+Na] <sup>1+</sup>	0.94
			138.05463	26472	138.05496	C7H7NO2	4-Amino benzoate	[M+H] <sup>1+</sup>	-2.36
			138.06632	4438	138.06619	C6H7N3O	6-Amino nicotinamide	[M+H] <sup>1+</sup>	0.95
			138.09106	3707	138.09134	C8H11NO	Tyramine	[M+H] <sup>1+</sup>	-2.03
E114F00h~E333F00h	139	Positive	139.03864	1298	139.03897	C7H6O3	Salicylate	[M+H] <sup>1+</sup>	-2.39
			139.04997	6643	139.05021	C6H6N2O2	Urocanate	[M+H] <sup>1+</sup>	-1.69
			139.06314	5781	-	-	Unknown	-	-
			139.08628	1429	139.08659	C7H10N2O	4-Hydroxymethyl phenyl hydrazine	[M+H] <sup>1+</sup>	-2.23
			140.06789	56514	140.06820	C5H11NO2	Betaine	[M+Na] <sup>1+</sup>	-2.21
E160F00h~E260F00h 2N=10~2N=20	141 141.9	Positive	141.00604	1011	141.00609	C3H6N2O2	N-Formimino glycine	[M+K] <sup>1+</sup>	-0.33
			141.07129	1728	-	-	<sup>13</sup> C Isotope of Betaine	[M+Na] <sup>1+</sup>	-
			142.02611	662	142.02649	C4H9NO2	γ-Amino butyrate	[M+K] <sup>1+</sup>	-2.66

E137F00h~E160F00h	144	Positive	142.03462	287089	142.03462	CH2O3	Carbonate	[2M+NH4]1+	0.03
			142.04697	706	142.04747	C4H9NO3	Threonine	[M+Na]1+	-3.48
			142.09690	1118	142.09749	C6H11N3O	Histidinol	[M+H]1+	-4.15
			144.00543	2431	144.00575	C3H7NO3	Serine	[M+K]1+	-2.24
			144.03214	27607	144.03251	C2H6O4S	2-Hydroxy ethane sulfonate	[M+NH4]1+	-2.57
			144.06523	1004	144.06552	C6H11NO4	2-Amino adipate	[M+H-H2O]1+	-2.02
E115F00h~E333F00h	145	Positive	144.07818	1793	144.07837	C8H11N	2,4-Dimethyl aniline	[M+Na]1+	-1.32
			144.08048	5912	144.08078	C10H9N	3-Methyl quinoline	[M+H]1+	-2.05
			144.10153	1015	144.10191	C7H13NO2	Proline Betaine	[M+H]1+	-2.61
			144.99260	2258	144.99299	C3H6O3S	3-Mercapto lactate	[M+Na]1+	-2.69
			145.02584	2258	145.02600	C7H6O2	Benzoate	[M+Na]1+	-1.11
			145.04925	1706	145.04954	C6H8O4	2,3-Dimethyl maleate	[M+H]1+	-1.98
2N=10~2N=30	147.09	Positive	145.08565	518	145.08592	C7H12O3	5-Aceto valerate	[M+H]1+	-1.87
			147.07615	144700	147.07642	C5H10N2O3	Glutamine	[M+H]1+	-1.84
			147.11243	3326	147.11281	C6H14N2O2	Lysine	[M+H]1+	-2.55
2N=20~2N=30	149.54	Positive	149.51780	619	149.51854	C11H11NO6	N-Pyruvoyl-5-methoxy-3-hydroxy anthranilate	[M+2Na]2+	-4.96
E115F00h~E129F00h	150	Positive	150.02434	8246	-	-	Unknown	-	-
E114F00h~E176F00h	156	Positive	150.05205	1418	150.05255	C6H9NO2	N-Ethyl succinimide	[M+Na]1+	-3.33
			150.05785	1306	150.05833	C5H11NO2S	Methionine	[M+H]1+	-3.20
			150.07716	1562	150.07742	C6H7N5	1-Methyl adenine	[M+H]1+	-1.75
			156.04174	2193725	156.04214	C5H11NO2	Betaine	[M+K]1+	-2.54
E114F00h~E176F00h	157	Positive	157.01040	4940	157.01075	C4H6O5	Malate	[M+Na]1+	-2.20
E114F00h~E176F00h	158	Positive	157.04508	104241	-	-	13C Isotope of Betaine	[M+K]1+	-
			158.03993	147095	-	-	41K Isotope of Betaine	[M+K]1+	-
			159.00825	3883	159.00864	C4H8O3S	Methyl-1-thio-glycerate	[M+Na]1+	-2.45
			159.02003	1170	159.02056	C6H7O3P	Phenyl phosphonate	[M+H]1+	-3.33
E114F00h~E162F00h	159	Positive	159.04318	4912	-	-	41K Isotope of 13C isotope of Betaine	[M+K]1+	-
			159.06298	1940	159.06278	C5H12O4	Apiitol	[M+Na]1+	1.25
			159.06496	827	159.06519	C7H10O4	2-Isopropyl maleate	[M+H]1+	-1.43
			159.07608	4776	159.07642	C6H10N2O3	4-Methylene glutamine	[M+H]1+	-2.14
			160.03656	1355	160.03690	C7H7NO2	4-Amino benzoate	[M+Na]1+	-2.12
			160.06008	1272	160.06044	C6H9NO4	N-Methyl-2-oxoglutaramate	[M+H]1+	-2.22
E480F00h~E485F00h	160	Positive	160.07530	1823	160.07569	C10H9NO	Indole-3-acetaldehyde	[M+H]1+	-2.44

E114F00h~E160F00h	161	Positive	160.08147	15032	-	-	Unknown	-	-
			160.09642	580	160.09682	C7H13NO3	3-Dehydro carnitine	[M+H]1+	-2.50
			160.99587	1006	160.99592	CH3NO2	Carbamate	[2M+K]1+	-0.29
2N=20~2N=30	173	Positive	161.05636	1703	161.05569	C5H8N2O4	N-Formimino aspartate	[M+H]1+	4.19
			161.09172	15738	161.09207	C6H12N2O3	Alanyl alanine	[M+H]1+	-2.17
			161.10691	2696	161.10733	C10H12N2	Tryptamine	[M+H]1+	-2.58
2N=20~2N=30	175	Positive	172.98404	10630	172.98468	C4H6O5	Malate	[M+K]1+	-3.72
			173.02061	987	173.02096	C3H9O6P	Glycerol 3-phosphate	[M+H]1+	-1.99
			173.05689	5698	173.05730	C9H10O2	4-Coumaryl alcohol	[M+Na]1+	-2.37
2N=20~2N=30	175	Positive	173.09153	1075	173.09207	C7H12N2O3	Glycyl proline	[M+H]1+	-3.12
			174.89537	108998	174.89594	H3O4P	Phosphate	[M+2K-H]1+	-3.25
			174.98220	1969	-	-	41K Isotope of Malate	[M+K]1+	-
E115F00h~E137F00h	176	Positive	174.99951	1388	175.00033	C4H8O5	Erythronate	[M+K]1+	-4.71
			175.10717	2176	175.10772	C7H14N2O3	N-Acetyl ornithine	[M+H]1+	-3.14
			175.11843	11805	175.11895	C6H14N4O2	Arginine	[M+H]1+	-2.99
E115F00h~E129F00h	182	Positive	176.01021	48076	176.01084	C7H7NO2	2-Amino benzoate	[M+K]1+	-3.57
			176.02235	1109	176.02207	C6H7N3O	6-Amino nicotinamide	[M+K]1+	1.59
			176.05801	10255	176.05846	C7H11N3	2,4,6-Triamino toluene	[M+K]1+	-2.53
E114F00h~E162F00h	183.09	Positive	176.09119	2036	176.09174	C7H13NO4	$\alpha$ -Amino pimelate	[M+H]1+	-3.10
			176.10230	16727	176.10297	C6H13N3O3	Argininate	[M+H]1+	-3.80
			182.08037	1542	182.08117	C9H11NO3	Tyrosine	[M+H]1+	-4.40
E114F00h~E137F00h	184	Positive	182.94488	2026	182.94565	C3H6O4	Glycerate	[M+2K-H]1+	-4.21
			183.06088	49094	-	-	Unknown	-	-
			183.10088	1107	183.10157	C10H14O3	Dihydro coniferyl alcohol	[M+H]1+	-3.78
2N=10~2N=20	185.09	Positive	183.11210	1295	183.11281	C9H11NO2	Phenylalanine	[M+NH4]1+	-3.85
			184.03635	2037	184.03705	C6H11NO3	5-Hydroxy pipecolate	[M+K]1+	-3.81
			184.05752	738	184.05803	C6H11NO4	2-Amino adipate	[M+Na]1+	-2.77
2N=10~2N=20	185.09	Positive	184.06423	2412	184.06381	C5H13NO4S	Choline sulfate	[M+H]1+	2.28
			184.07264	3572	184.07344	C7H15NO2	3-Dehydroxy carnitine	[M+K]1+	-4.34
			185.00535	8688	185.00566	C5H6O6	4-Hydroxy-2-oxoglutarate	[M+Na]1+	-1.68
2N=10~2N=20	185.09	Positive	185.02027	957	185.02107	C6H10O4	Adipate	[M+K]1+	-4.32
			185.03161	192517	185.03230	C5H10N2O3	Glutamine	[M+K]1+	-3.73
			185.04130	1213	185.04205	C6H10O5	3,3-Dimethyl malate	[M+Na]1+	-4.03

E115F00h~E137F00h	187.09	Positive	185.06809	1045	185.06869	C6H14N2O2	Lysine	[M+K]1+	-3.23
			186.99946	1498	187.00022	C3H7O7P	3-Phosphoglycerate	[M+H]1+	-4.06
			187.02959	6927	-	-	41K Isotope of Glutamine	[M+K]1+	-
<b>Pilar analysis</b>	<b>188.09</b>	<b>Positive</b>	187.03591	1520	187.03657	C9H8O3	<i>p</i> -Coumarate	[M+Na]1+	-3.51
			<b>188.01373</b>	<b>2659</b>	<b>188.01421</b>	<b>C5H11NO2S</b>	<b>L-Methionine</b>	<b>[M+K]1+</b>	<b>-2.56</b>
			<b>188.06743</b>	<b>918</b>	<b>188.06820</b>	<b>C9H11NO2</b>	<b>L-Phenylalanine</b>	<b>[M+Na]1+</b>	<b>-4.10</b>
E114F00h~E115F00h	191	Positive	<b>190.97868</b>	<b>13086</b>	<b>190.97883</b>	<b>C5H4N4S</b>	<b>6-Mercaptopurin</b>	<b>[M+K]1+</b>	<b>-0.77</b>
			<b>191.03095</b>	<b>1121</b>	<b>191.03163</b>	<b>C5H12O5</b>	<b>Xylitol</b>	<b>[M+K]1+</b>	<b>-3.58</b>
			<b>191.10584</b>	<b>1280</b>	<b>191.10666</b>	<b>C12H14O2</b>	<b>Propyl cinnamate</b>	<b>[M+H]1+</b>	<b>-4.28</b>
2N=10~2N=20	194.09	Positive	193.98188	1153	193.98250	C2H6NO6P	Phosphoglycolo hydroxamate	[M+Na]1+	-3.19
			<b>194.05861</b>	<b>2340</b>	<b>194.05779</b>	<b>C8H13NO2</b>	<b>Scopoline</b>	<b>[M+K]1+</b>	<b>4.24</b>
			<b>194.94495</b>	<b>1000</b>	<b>194.94554</b>	<b>C2H5O6P</b>	<b>2-Phospho glycolate</b>	<b>[M+K]1+</b>	<b>-3.01</b>
E114F00h~E480F00h	195.09	Positive	<b>195.04121</b>	<b>1120</b>	<b>195.04180</b>	<b>C8H12O3</b>	<b>5-Oxo-7-octenoate</b>	<b>[M+K]1+</b>	<b>-3.04</b>
			<b>195.07739</b>	<b>1148</b>	<b>195.07819</b>	<b>C9H16O2</b>	<b>2-Nonenoate</b>	<b>[M+K]1+</b>	<b>-4.10</b>
			<b>195.08573</b>	<b>1539</b>	<b>195.08632</b>	<b>C7H14O6</b>	<b>Methyl glucoside</b>	<b>[M+H]1+</b>	<b>-3.01</b>
			<b>195.09854</b>	<b>1007</b>	<b>195.09917</b>	<b>C9H16O3</b>	<b>3-Caproyl propionate</b>	<b>[M+Na]1+</b>	<b>-3.21</b>
			203.05182	2016	203.05261	C6H12O6	Glucose	[M+Na]1+	-3.90
2N=10~2N=30	208.09	Positive	207.99759	1383	207.99815	C3H8NO6P	<i>O</i> -Phospho serine	[M+Na]1+	-2.68
E160F00h~E260F00h	210	Positive	<b>208.04799</b>	<b>2098</b>	<b>208.04829</b>	<b>C7H11N3O2</b>	<b>1- or 3-Methyl histidine</b>	<b>[M+K]1+</b>	<b>-1.42</b>
			<b>209.95588</b>	<b>9407</b>	<b>209.95644</b>	<b>C2H6NO6P</b>	<b>Phosphoglycolo hydroxamate</b>	<b>[M+K]1+</b>	<b>-2.64</b>
			<b>210.05198</b>	<b>1232</b>	<b>210.05255</b>	<b>C11H9NO2</b>	<b>Indole acrylate</b>	<b>[M+Na]1+</b>	<b>-2.71</b>
2N=10~2N=30	211.09	Positive	<b>210.93987</b>	<b>26857</b>	-	-	<b>Unknown</b>	-	-
			<b>211.03597</b>	<b>1135</b>	<b>211.03672</b>	<b>C8H12O4</b>	<b>4-Octenedioate</b>	<b>[M+K]1+</b>	<b>-3.55</b>
			<b>211.04879</b>	<b>1421</b>	<b>211.04795</b>	<b>C7H12N2O3</b>	<b>Glycyl proline</b>	<b>[M+K]1+</b>	<b>3.97</b>
			<b>211.07240</b>	<b>3275</b>	<b>211.07310</b>	<b>C9H16O3</b>	<b>6-Propionyl N-caproate</b>	<b>[M+K]1+</b>	<b>-3.34</b>
			211.09351	612	211.09408	C9H16O4	Azelaic acid	[M+Na]1+	-2.70
E114F00h~E160F00h	214.09	Positive	211.13208	802	211.13287	C12H18O3	Jasmonate	[M+H]1+	-3.75
			<b>214.04679</b>	<b>1746</b>	<b>214.04747</b>	<b>C10H9NO3</b>	<b>Indole-3-glycolate</b>	<b>[M+Na]1+</b>	<b>-3.15</b>
			<b>214.05817</b>	<b>49449</b>	<b>214.05885</b>	<b>C6H13N3O3</b>	<b>Citrulline</b>	<b>[M+K]1+</b>	<b>-3.18</b>
E129F00h~E137F00h	215	Positive	<b>215.01556</b>	<b>8683</b>	<b>215.01623</b>	<b>C6H8O7</b>	<b>2-Keto-3-deoxy glucarate</b>	<b>[M+Na]1+</b>	<b>-3.10</b>
			<b>215.03094</b>	<b>1850</b>	<b>215.03163</b>	<b>C7H12O5</b>	<b>2-Isopropyl malate</b>	<b>[M+K]1+</b>	<b>-3.22</b>
			<b>215.05057</b>	<b>1744</b>	<b>215.05026</b>	<b>C8H16O2S</b>	<b>6-Thio-octanoate</b>	<b>[M+K]1+</b>	<b>1.43</b>
E129F00h~E480F00h	217.18	Positive	<b>217.01024</b>	<b>5010</b>	<b>217.01079</b>	<b>C4H9O8P</b>	<b>Erythronate-4-phosphate</b>	<b>[M+H]1+</b>	<b>-2.51</b>

E115F00h~E480F00h	219.09	Positive	217.03135	10870	217.03188	C6H10O7	Glucuronate	[M+Na]1+	-2.42
			219.02584	46171	219.02655	C6H12O6	Glucose	[M+K]1+	-3.23
E137F00h~E176F00h	224.09	Positive	223.97144	18021	223.97209	C3H8NO6P	Phospho serine	[M+K]1+	-2.88
E176F00h~E480F00h	225	Positive	224.07594	22054	224.07648	C7H10O7	Homocitrate	[M+NH4]1+	-2.41
			224.95539	1040	224.95610	C3H7O7P	3-Phospho glycerate	[M+K]1+	-3.16
E115F00h~E137F00h	227.09	Positive	225.05162	2070	225.05237	C9H14O4	2-Carboxy cyclohexyl acetate	[M+K]1+	-3.33
			225.06340	1093	225.06345	C11H10N2O2	Didehydro tryptophan	[M+Na]1+	-0.22
E114F00h~E480F00h	229.18	Positive	225.07447	2789	225.07335	C9H14O5	Diethyl 2-methyl-3-oxosuccinate	[M+Na]1+	4.99
			225.08794	1523	225.08699	C10H12N2O4	3-Hydroxy kynurenine	[M+H]1+	4.24
2N=10~2N=30	231.09	Positive	225.09912	1113	225.09849	C10H12N5	N6-Dimethyl allyl adenine	[M+Na]1+	2.79
			225.14784	1650	225.14852	C13H20O3	Vomifolol	[M+H]1+	-3.03
2N=10~2N=20	233.18	Positive	226.99449	837	226.99525	C7H8O6	Homoaconitate	[M+K]1+	-3.34
			227.01534	2207	227.01623	C7H8O7	Oxaloglutarate	[M+Na]1+	-3.90
2N=20~2N=30	235.09	Positive	227.03089	1265	227.03163	C8H12O5	2-Oxo suberate	[M+K]1+	-3.27
			227.04211	901	227.04287	C7H12N2O4	N-Acetyl glutamine	[M+K]1+	-3.33
2N=10~2N=20	233.18	Positive	227.06706	1584	227.06802	C9H16O4	Azelaic acid	[M+K]1+	-4.22
			227.07882	1519	227.07910	C11H12N2O2	Tryptophan	[M+Na]1+	-1.23
2N=10~2N=30	231.09	Positive	229.03122	984	229.03188	C7H10O7	Homocitrate	[M+Na]1+	-2.86
			229.04637	1270	229.04717	C6H13O7P	Mevalonate-5P	[M+H]1+	-3.49
2N=10~2N=20	233.18	Positive	229.05803	1061	229.05852	C7H14N2O4	Diamino pimelate	[M+K]1+	-2.12
			229.14253	1935	229.14344	C12H20O4	Traumatate	[M+H]1+	-3.96
2N=20~2N=30	235.09	Positive	230.98947	75153	230.99016	C6H8O7	2,5-Didehydro gluconate	[M+K]1+	-3.00
			231.02564	5353	231.02655	C7H12O6	Quinate	[M+K]1+	-3.93
2N=10~2N=20	233.18	Positive	231.06335	1208	231.06278	C11H12O4	Sinapaldehyde	[M+Na]1+	2.46
			231.09853	808	231.09755	C9H14N2O5	Aspartyl proline	[M+H]1+	4.24
2N=20~2N=30	235.09	Positive	231.13499	877	231.13394	C5H9NO2	Proline	[2M+H]1+	4.56
			232.98746	3254	-	-	41K Isotope of 2,5-Didehydro gluconate	[M+K]1+	-
2N=10~2N=20	233.18	Positive	233.00481	978	233.00581	C6H10O7	Glucuronate	[M+K]1+	-4.30
			233.04129	1727	233.04205	C10H10O5	2-Hydroxy ferulate	[M+Na]1+	-3.24
2N=20~2N=30	235.09	Positive	233.07766	1432	233.07843	C11H14O4	Sinapyl Alcohol	[M+Na]1+	-3.31
			235.02053	2221	235.02146	C6H12O7	Gluconate	[M+K]1+	-3.97
2N=20~2N=30	235.09	Positive	235.07232	13897	235.07295	C14H12O2	4,4'-Dihydroxy stilbene	[M+Na]1+	-2.68
			235.16433	32627	235.16524	C5H11NO2	Valine	[2M+H]1+	-3.85

E137F00h~E162F00h	236.09	Positive	236.03254	1383	236.03197	C9H11NO4	DOPA	[M+K]1+	2.42
			236.04339	1387	236.04320	C8H11N3O3	Acetyl histidine	[M+K]1+	0.80
			236.07573	1054	236.07648	C8H13NO7	N-Acetyl galactosamine	[M+H]1+	-3.18
2N=20~2N=30	237.09	Positive	237.05138	1440	237.05222	C13H10O3	Phenyl salicylate	[M+Na]1+	-3.53
			237.08795	4236	237.08699	C11H12N2O4	N-Formyl kynurenine	[M+H]1+	4.07
			237.14798	1584	237.14852	C14H20O3	4-Heptyl oxybenzoate	[M+H]1+	-2.29
2N=10~2N=20	241	Positive	240.86532	135571	-	-	Unknown	-	-
			241.04647	1367	241.04728	C9H14O5	Diethyl-2-methyl-3-oxosuccinate	[M+K]1+	-3.37
			241.08285	1779	241.08367	C10H18O4	Sebacate	[M+K]1+	-3.39
			241.09412	7711	241.09475	C12H14N2O2	Acetyl serotonin	[M+Na]1+	-2.61
			241.15383	1693	241.15467	C12H20N2O3	Slaframine	[M+H]1+	-3.48
2N=20~2N=30	241.72	Positive	241.58886	1724	241.58844	C21H31N3O5S	FMLP	[M+2Na]2+	1.72
2N=10~2N=20	243	Positive	242.98919	7016	242.99016	C7H8O7	Oxaloglutamate	[M+K]1+	-4.00
			243.01001	1324	243.01114	C7H8O8	4-Oxalo citramalate	[M+Na]1+	-4.65
			243.04499	688	-	-	41K Isotope of Diethyl-2-methyl-3-oxosuccinate	[M+K]1+	-
			243.05196	1314	243.05304	C11H12N2O2	Tryptophan	[M+K]1+	-4.43
2N=20~2N=30	244.18	Positive	244.02103	1183	244.02165	C10H7NO5	7,8-Dihydroxy kynurenate	[M+Na]1+	-2.52
			244.05712	1063	244.05818	C8H15NO5	N-Acetyl-fucosamine	[M+K]1+	-4.35
			244.09365	2547	244.09457	C9H19NO4	Pantothenol	[M+K]1+	-3.76
2N=10~2N=30	245.09	Positive	244.98731	908	-	-	41K Isotope of Oxaloglutamate	[M+K]1+	-
			245.00482	22984	245.00581	C7H10O7	Homocitrate	[M+K]1+	-4.05
2N=10~2N=30	246.09	Positive	246.02763	1156	246.02755	C9H9N3O3	Methyl 5-hydroxy-2-benzimidazole carbamate	[M+K]1+	0.33
E160F00h~E176F00h	249.09	Positive	249.03617	3231	249.03696	C10H10O6	Chorismate	[M+Na]1+	-3.18
			249.08800	1382	249.08875	C12H18O3	Jasmonate	[M+K]1+	-3.02
E137F00h~E160F00h	251.09	Positive	251.06733	1327	251.06787	C14H12O3	Resveratrol	[M+Na]1+	-2.13
			251.09430	1157	251.09451	C14H16N2	3,3'-Dimethyl benzidine	[M+K]1+	-0.82
			251.10356	4929	251.10440	C12H20O3	12-Oxo-10-dodecenoate	[M+K]1+	-3.36
			251.14027	1008	251.13902	C13H18N2O3	N-Caffeoyl putrescine	[M+H]1+	4.98
2N=10~2N=30	255.18	Positive	255.02727	763	255.02644	C7H11O8P	Shikimate-3-phosphate	[M+H]1+	3.27
			255.08606	1175	255.08540	C8H16N4O3	N-α-Acetyl arginine	[M+K]1+	2.59
			255.09846	1538	255.09932	C11H20O4	Undecane dioate	[M+K]1+	-3.37
			255.10672	909	255.10745	C9H18O8	Galactosyl glycerol	[M+H]1+	-2.85
			255.13509	1251	255.13570	C12H24O3	α-Hydroxy laurate	[M+K]1+	-2.41



2N=20~2N=30	257.08	Positive	257.05297	1020	257.05343	C8H14N2O5	$\gamma$ -Glutamyl alanine	[M+K]1+	-1.79
			257.07753	1959	257.07858	C10H18O5	3-Hydroxy sebacate	[M+K]1+	-4.10
			257.14639	3440	257.14718	C5H11NO2	Valine	[2M+Na]1+	-3.07
E162F00h~E176F00h 2N=10~2N=30	258.18 259	Positive	258.07327	1410	258.07383	C9H17NO5	Pantothenate	[M+K]1+	-2.18
			259.02067	3169	259.02146	C8H12O7	(Homo)2-citrate	[M+K]1+	-3.06
			259.05729	1131	259.05770	C12H12O5	5,6,7-Trimethoxy coumarin	[M+Na]1+	-1.57
E114F00h~E480F00h	261.18	Positive	259.12920	1059	259.13047	C14H20O3	Heptyl <i>p</i> -hydroxybenzoate	[M+Na]1+	-4.89
			261.01874	1463	-	-	41K Isotope of (Homo)2-citrate	[M+K]1+	-
			261.03641	1046	261.03700	C6H13O9P	Glucose 6-phosphate	[M+H]1+	-2.26
E333F00h~E480F00h	263.08	Positive	261.05143	9757	261.05237	C12H14O4	Coniferyl acetate	[M+K]1+	-3.60
			261.07256	1453	261.07335	C12H14O5	3,4,5-Trimethoxy cinnamate	[M+Na]1+	-3.01
			261.08803	1824	261.08875	C13H18O3	Dehydro vomifoliol	[M+K]1+	-2.77
E137F00h~E162F00h 2N=20~2N=30	264.18 268.18	Positive	263.04952	2127	-	-	41K Isotope of Coniferyl acetate	[M+K]1+	-
			263.10365	18497	263.10440	C13H20O3	Vomifoliol	[M+K]1+	-2.86
			264.10693	2008	-	-	13C Isotope of Vomifoliol	[M+K]1+	-
E129F00h~E137F00h	269.18	Positive	268.10297	10555	268.10403	C10H13N5O4	Adenosine	[M+H]1+	-3.96
			268.13880	1059	268.13801	C9H19N5O3	$\beta$ -Alanyl arginine	[M+Na]1+	2.94
			268.98160	3680	268.98232	C5H11O8P	Ribose-5-phosphate	[M+K]1+	-2.67
2N=10~2N=30	271.08	Positive	269.08883	899	269.08982	C5H9NO2	Proline	[2M+K]1+	-3.66
			269.09649	1194	269.09609	C20H12O	2-Hydroxybenzo[ <i>a</i> ]pyrene	[M+H]1+	1.48
			269.11413	1568	269.11497	C12H22O4	Diisopropyl adipate	[M+K]1+	-3.11
E114F00h~E176F00h E162F00h~E333F00h	273.18 275.08	Positive	271.07624	1348	271.07648	C11H20O3S	2-Oxo-10-methyl thiodecanoate	[M+K]1+	-0.88
			271.08316	1012	271.08434	C13H16N2O2	Melatonin	[M+K]1+	-4.34
			271.09335	1554	271.09408	C14H16O4	Prenyl caffeate	[M+Na]1+	-2.70
E129F00h~E137F00h	277.18	Positive	273.12024	46230	273.12112	C5H11NO2	Valine	[2M+K]1+	-3.21
			275.01541	11332	275.01627	C6H11O10P	Glucuronate 1-phosphate	[M+H]1+	-3.11
			275.10345	1355	275.10264	C7H7NO2	<i>p</i> -Amino benzoate	[2M+H]1+	2.96
E129F00h~E137F00h	278.18	Positive	275.11842	2918	-	-	41K Isotope of Valine	[2M+K]1+	-
			277.03127	71614	277.03203	C8H14O8	3-Deoxy octulosonate	[M+K]1+	-2.74
			277.11937	4202	277.12005	C14H22O3	1 $\alpha$ ,1 $\beta$ -Dihomo jasmonate	[M+K]1+	-2.46
E129F00h~E137F00h	279.18	Positive	277.14024	1156	277.13942	C11H20N2O6	Saccharopine	[M+H]1+	2.98
			278.03456	3855	-	-	13C Isotope of 3-Deoxy octulosonate	[M+K]1+	-
			279.02923	2862	-	-	41K Isotope of 3-Deoxy octulosonate	[M+K]1+	-

2N=10~2N=20	282.18	Positive	279.04695	3228	279.04768	C4H8O4	Erythrose	[2M+K]1+	-2.61
			279.05510	12070	-	-	Unknown	-	-
			279.09858	5363	279.09917	C16H16O3	Pterostilbene	[M+Na]1+	-2.10
			279.11039	1143	279.11055	C12H20N2O3	Slaframine	[M+K]1+	-0.58
			282.11119	938	282.11022	C12H21NO4	Tiglyl carnitine	[M+K]1+	3.45
E129F00h~E480F00h	283.18	Positive	282.14585	8763	282.14483	C13H19N3O4	Pendimethalin	[M+H]1+	3.60
			282.27856	935	282.27914	C18H35NO	Oleamide	[M+H]1+	-2.06
			283.01969	988	283.01894	C6H13O9P	Glucose 6-phosphate	[M+Na]1+	2.64
			283.12986	1123	283.13062	C13H24O4	2-Methyl-dodecanedioate	[M+K]1+	-2.68
			283.26204	1007	283.26316	C18H34O2	Oleate	[M+H]1+	-3.94
E115F00h~E129F00h	286.08	Positive	286.07658	881	286.07688	C4H6O5	Malate	[2M+NH4]1+	-1.03
			286.11236	899	286.11326	C9H16O9	Glucosyl glycerate	[M+NH4]1+	-3.15
			286.96626	1239	286.96648	C3H8OS2	6-Acetyl dihydrolipoate	[2M+K]1+	-0.78
			287.00047	1502	287.00097	C4H4O5	Oxaloacetate	[2M+Na]1+	-1.75
			287.14064	1344	287.14079	C16H24O2	8,10-Hexadeca diynoate	[M+K]1+	-0.52
E162F00h~E485F00h	294.18	Positive	287.16106	4540	287.16177	C16H24O3	Dehydro juvabione	[M+Na]1+	-2.46
			294.06596	1051	-	-	Unknown	-	-
			294.07304	992	294.07368	C15H13NO4	Benzoyl-4-methoxy anthranilate	[M+Na]1+	-2.18
			294.11756	1187	294.11835	C11H19NO8	N-Acetyl muramate	[M+H]1+	-2.67
			295.11245	1651	295.11360	C5H9NO4	Glutamate	[2M+H]1+	-3.88
E333F00h~E480F00h	295.18	Positive	295.12957	2824	295.12885	C14H18N2O5	Glutamyl phenylalanine	[M+H]1+	2.44
			296.06510	14680	296.06599	C8H20NO6P	Glycero phosphocholine	[M+K]1+	-2.99
			296.12753	1655	296.12812	C18H17NO3	Xylopin	[M+H]1+	-2.00
			298.99236	786	298.99288	C6H13O9P	Glucose 6-phosphate	[M+K]1+	-1.75
			299.08852	1486	299.08915	C6H10O3	2-Keto-N-caproate	[2M+K]1+	-2.10
E114F00h~E160F00h	299.18	Positive	303.00705	1119	303.06631	C11H20O5S	2-(6'-Methylthio)hexylmalate	[M+K]1+	-195.52
			303.08325	20028	-	-	Unknown	-	-
			303.09842	1223	303.09932	C15H20O4	Abscisate	[M+K]1+	-2.96
			307.04192	967	307.04259	C9H16O9	Glucosyl glycerate	[M+K]1+	-2.20
			307.07813	1430	307.07898	C5H10O4	Deoxy ribose	[2M+K]1+	-2.76
2N=10~2N=30	307.18	Positive	307.09341	1005	307.09408	C17H16O4	Phenethyl caffeate	[M+Na]1+	-2.18
			307.20234	1025	307.20339	C17H32O2	3-Hepta decenoate	[M+K]1+	-3.41
			311.89147	4346	-	-	Unknown	-	-

E137F00h~E480F00h	313.18	Positive	312.22905	893	312.22994	C16H35NO2	C16 Sphinganine	[M+K]1+	-2.84
			313.02811	1026	313.02951	C7H15O10P	Sedoheptulose 7-phosphate	[M+Na]1+	-4.47
			313.09405	4456	313.09490	C15H18N2O3	Indole-3-acetyl-valine	[M+K]1+	-2.72
			313.11525	3049	313.11588	C15H18N2O4	6-Hydroxy-indole-3-acetyl-valine	[M+Na]1+	-2.01
E129F00h~E162F00h	314	Positive	313.15545	2614	313.15644	C18H26O2	17-Octadecene-9,11-Diynoate	[M+K]1+	-3.15
			314.12283	1118	314.12343	C14H19NO7	Tyramine glucuronide	[M+H]1+	-1.91
			314.15923	1439	314.15982	C15H20O6	Vomitoxin	[M+NH4]1+	-1.87
			314.98717	1328	314.98780	C6H13O10P	6-Phospho gluconate	[M+K]1+	-1.99
E129F00h~E160F00h	315	Positive	315.08290	1015	315.08391	C15H16O6	Dihydro mikanolide	[M+Na]1+	-3.21
			315.13468	1242	315.13570	C17H24O3	[6]-Shogaol	[M+K]1+	-3.25
			316.16621	763	316.16734	C17H27NO2	Venlafaxine	[M+K]1+	-3.56
			316.21053	725	316.21185	C16H29NO5	Butoctamide hydrogen succinate	[M+H]1+	-4.18
E333F00h~E480F00h	319.18	Positive	319.09611	1010	319.09649	C20H14O4	8-O-Methyl tetragulol	[M+H]1+	-1.18
			319.11456	3308	319.11521	C15H20O6	Vomitoxin	[M+Na]1+	-2.04
			319.12972	1300	319.13047	C19H20O3	4-Prenyl resveratrol	[M+Na]1+	-2.34
			320.04634	1171	320.04658	C12H15N3O3S	Albendazole oxide	[M+K]1+	-0.73
E114F00h~E176F00h	320.18	Positive	320.11783	1115	320.11935	C14H23N3OS	Xanomeline	[M+K]1+	-4.73
			320.23404	2395	320.23502	C18H35NO	Oleamide	[M+K]1+	-3.07
			322.98018	841	322.98087	C6H6O2S	Thien-2-ylacetate	[2M+K]1+	-2.12
			323.07295	1418	323.07374	C13H16O8	4-(β-Glucosyloxy)benzoate	[M+Na]1+	-2.45
E129F00h~E137F00h	323.18	Positive	323.08833	1189	323.08738	C14H14N2O7	3-Hydroxy-2-oxindole-3-acetyl-aspartate	[M+H]1+	2.94
			323.12456	1345	323.12538	C18H20O4	Toxyl angelate	[M+Na]1+	-2.54
			323.14496	628	323.14490	C6H11NO4	2-Amino adipate	[2M+H]1+	0.20
			326.10752	561	326.10818	C11H19NO10	N-Glycolyl neuraminate	[M+H]1+	-2.01
E114F00h~E137F00h	326	Positive	326.14338	1652	-	-	Unknown	-	-
			326.17998	1695	326.17845	C17H27NO3S	Cycloxydim	[M+H]1+	4.71
			329.00396	571	329.00345	C7H15O10P	Sedoheptulose 7-phosphate	[M+K]1+	1.56
			329.13524	1005	329.13595	C17H22O5	Xanthinin	[M+Na]1+	-2.15
2N=10~2N=30	329.18	Positive	329.15046	5330	329.15135	C18H26O3	8-Oxo-9,11-octadecadiynoate	[M+K]1+	-2.72
			329.17147	600	329.17233	C18H26O4	Di-N-Pentyl phthalate	[M+Na]1+	-2.62
			341.81673	41617	-	-	Unknown	-	-
			342.13892	1295	342.13948	C12H23NO10	6-(α-Glucosaminyl)-1myo-inositol	[M+H]1+	-1.63
E176F00h~E480F00h	343.18	Positive	343.07810	4656	343.07883	C16H16O7	p-Coumaroyl shikimate	[M+Na]1+	-2.12

E114F00h~E480F00h	345.18	Positive	343.11495	1552	343.11360	C7H9NO4	5-Deoxy-5-amino-3-dehydroshikimate	[2M+H] <sup>1+</sup>	3.95
			343.12417	590	343.12349	C12H22O11	Sucrose	[M+H] <sup>1+</sup>	1.98
			345.09400	15428	-	-	Unknown	-	-
E114F00h~E333F00h	351.18	Positive	345.11969	1719	345.12096	C19H18N2O3	<i>p</i> -Coumaroyl serotonin	[M+Na] <sup>1+</sup>	-3.69
			345.14563	2180	345.14627	C18H26O4	Dipentyl phthalate	[M+K] <sup>1+</sup>	-1.85
			351.04685	2791	351.04653	C6H16N4O9P2	N-Phospho lombricine	[M+H] <sup>1+</sup>	0.90
2N=10~2N=20	355.18	Positive	351.06790	1956	351.06881	C11H20O10	Arabino galactose	[M+K] <sup>1+</sup>	-2.59
			351.08314	1249	351.08406	C15H20O7	4-Hydroxycinnamyl alcohol 4-glucoside	[M+K] <sup>1+</sup>	-2.63
			351.22849	1113	351.22960	C19H36O3	10-Oxo-nonadecanoate	[M+K] <sup>1+</sup>	-3.17
E114F00h~E480F00h	357.27	Positive	355.07808	3747	355.07898	C7H10O4	2-Isopropyl maleate	[2M+K] <sup>1+</sup>	-2.53
			357.09376	2291	357.09448	C17H18O7	Byakangelicin	[M+Na] <sup>1+</sup>	-2.01
			357.11968	799	357.11801	C16H20O9	1-O-Feruloyl-β-glucose	[M+H] <sup>1+</sup>	4.67
2N=10~2N=20	359.18	Positive	357.16688	1099	357.16740	C16H30O6	Menthyl O-β-Glucoside	[M+K] <sup>1+</sup>	-1.45
			358.99200	6133	358.99376	C12H17O4PS2	Phenthoate	[M+K] <sup>1+</sup>	-4.89
			359.09880	853	359.10027	C14H19N2O7P	α-Ribazole-5'-P	[M+H] <sup>1+</sup>	-4.09
2N=20~2N=30	364.09	Positive	359.10943	5379	359.11028	C14H24O8	Valproate glucuronide	[M+K] <sup>1+</sup>	-2.36
			359.12428	757	359.12377	C9H9NO3	Hippurate	[2M+H] <sup>1+</sup>	1.43
			359.14595	1388	359.14623	C16H18N6O4	2-Phenyl amino adenosine	[M+H] <sup>1+</sup>	-0.78
E114F00h~E162F00h	365.27	Positive	364.12285	1440	364.12142	C12H23NO10	Lactosamine	[M+Na] <sup>1+</sup>	3.93
			364.26016	977	364.26124	C20H39NO2	N-Oleoyl ethanolamine	[M+K] <sup>1+</sup>	-2.96
			365.10457	21308	365.10544	C12H22O11	Sucrose	[M+Na] <sup>1+</sup>	-2.37
E114F00h~E162F00h	366.27	Positive	366.10794	2246	-	-	13C Isotope of Sucrose	[M+Na] <sup>1+</sup>	-
			366.96049	2007	366.95933	C7H15O10P	Sedoheptulose 7-phosphate	[M+2K-H] <sup>1+</sup>	3.17
			367.11496	995	367.11536	C16H24O7	Prostaglandin M	[M+K] <sup>1+</sup>	-1.10
E114F00h~E480F00h	371.27	Positive	367.18736	2032	367.18813	C18H32O5	9,10,11-Trihydroxy-12,15-octadecadienoate	[M+K] <sup>1+</sup>	-2.11
			367.22398	1011	367.22437	C22H32O3	7-Hydroxy-4Z,8E,10Z,13Z,16Z,19Z-docosahexenoic acid	[M+Na] <sup>1+</sup>	-1.05
			371.10953	10921	-	-	Unknown	-	-
E114F00h~E480F00h	373.27	Positive	371.14634	2118	371.14651	C19H24O6	Gibberellin A34	[M+Na] <sup>1+</sup>	-0.46
			373.01876	1381	373.01977	C11H15N2O8P	Nicotinamide ribotide	[M+K] <sup>1+</sup>	-2.69
			373.02897	900	373.02966	C9H19O11P	2-(Glucosyl)-sn-glycerol 3-phosphate	[M+K] <sup>1+</sup>	-1.85
E114F00h~E480F00h	373.27	Positive	373.12536	66710	-	-	Unknown	-	-
			373.15042	861	373.14931	C17H24O9	Syringin	[M+H] <sup>1+</sup>	2.97
			373.17715	2272	373.17757	C20H30O4	PGA2	[M+K] <sup>1+</sup>	-1.12

<b>E129F00h~E480F00h</b>	<b>374.18</b>	<b>Positive</b>	374.12897	6759	-	-	13C Isotope of 373.12536	-	-
			<b>374.28115</b>	<b>1397</b>	-	-	<b>Unknown</b>	-	-
<b>E114F00h~E480F00h</b>	<b>375.18</b>	<b>Positive</b>	<b>375.12268</b>	<b>3266</b>	<b>375.12367</b>	<b>C18H24O5S</b>	<b>Estradiol-17β 3-sulfate</b>	<b>[M+Na]1+</b>	<b>-2.64</b>
			<b>375.13106</b>	<b>5819</b>	<b>375.13153</b>	<b>C20H20N2O4</b>	<b>Feruloyl serotonin</b>	<b>[M+Na]1+</b>	<b>-1.25</b>
			<b>375.19291</b>	<b>1155</b>	<b>375.19322</b>	<b>C20H32O4</b>	<b>8,15-Dihydroxy-5,9,11,13-eicosatetraenoate</b>	<b>[M+K]1+</b>	<b>-0.82</b>
E129F00h~E137F00h	377.27	Positive	377.06240	1217	377.06333	C16H18O8	<i>p</i> -Coumaroyl quinate	[M+K]1+	-2.46
			377.07047	1148	377.07146	C7H8O6	Homoaconitate	[2M+H]1+	-2.62
<b>E114F00h~E137F00h</b>	<b>381.18</b>	<b>Positive</b>	<b>381.07882</b>	<b>821755</b>	<b>381.07937</b>	<b>C12H22O11</b>	<b>Sucrose</b>	<b>[M+K]1+</b>	<b>-1.45</b>
E114F00h~E137F00h	382.18	Positive	382.08183	75346	-	-	13C Isotope of Sucrose	[M+K]1+	-
E114F00h~E137F00h	383.18	Positive	383.07674	35850	-	-	41K Isotope of Sucrose	[M+K]1+	-
<b>2N=20~2N=30</b>	<b>384.18</b>	<b>Positive</b>	384.08042	3380	-	-	41K Isotope of 13C isotope of Sucrose	[M+K]1+	-
			<b>384.14973</b>	<b>664</b>	<b>384.15004</b>	<b>C14H25NO11</b>	<b>N-Acetyl lactosamine</b>	<b>[M+H]1+</b>	<b>-0.81</b>
			<b>384.29526</b>	<b>1198</b>	-	-	<b>Unknown</b>	-	-
<b>E129F00h~E480F00h</b>	<b>385.18</b>	<b>Positive</b>	<b>385.09929</b>	<b>1026</b>	<b>385.10078</b>	<b>C7H11NO4</b>	<b>5-Deoxy-5-aminoshikimate</b>	<b>[2M+K]1+</b>	<b>-3.86</b>
			<b>385.11394</b>	<b>771</b>	<b>385.11293</b>	<b>C17H20O10</b>	<b>O-Sinapoyl glucarolactone</b>	<b>[M+H]1+</b>	<b>2.63</b>
			<b>385.12525</b>	<b>4188</b>	<b>385.12578</b>	<b>C19H22O7</b>	<b>Vernolide</b>	<b>[M+Na]1+</b>	<b>-1.37</b>
			<b>385.16174</b>	<b>1610</b>	<b>385.16231</b>	<b>C17H30O7</b>	<b>3-Hydroxytetradecane-1,3,4-tricarboxylate</b>	<b>[M+K]1+</b>	<b>-1.49</b>
			<b>385.19862</b>	<b>1031</b>	<b>385.19870</b>	<b>C18H34O6</b>	<b>9,10-Dihydroxy octadecanedioate</b>	<b>[M+K]1+</b>	<b>-0.20</b>
<b>E114F00h~E480F00h</b>	<b>387.27</b>	<b>Positive</b>	387.10467	8699	387.10504	C9H10O4	Dihydro caffeate	[2M+Na]1+	-0.96
			<b>387.14070</b>	<b>10059</b>	<b>387.14158</b>	<b>C8H14O4</b>	<b>Suberate</b>	<b>[2M+K]1+</b>	<b>-2.27</b>
			<b>387.17738</b>	<b>1390</b>	<b>387.17781</b>	<b>C20H28O6</b>	<b>Gibberellin A37</b>	<b>[M+Na]1+</b>	<b>-1.11</b>
			<b>387.19307</b>	<b>837</b>	<b>387.19322</b>	<b>C21H32O4</b>	<b>17α,21-Dihydroxy pregnenolone</b>	<b>[M+K]1+</b>	<b>-0.38</b>
<b>E137F00h~E480F00h</b>	<b>389.18</b>	<b>Positive</b>	<b>389.00301</b>	<b>2661</b>	<b>389.00242</b>	<b>C6H16N4O9P2</b>	<b>N-Phospho lombricine</b>	<b>[M+K]1+</b>	<b>1.53</b>
			<b>389.08377</b>	<b>1109</b>	<b>389.08196</b>	<b>C18H22O5S</b>	<b>Estrone 3-sulfate</b>	<b>[M+K]1+</b>	<b>4.66</b>
			389.09439	1944	389.09259	C6H10O7	Glucuronate	[2M+H]1+	4.63
			389.10347	959	389.10229	C5H13NO4S	Choline sulfate	[2M+Na]1+	3.04
			<b>389.12027</b>	<b>18046</b>	<b>389.12156</b>	<b>C19H26O3S2</b>	<b>1α,5α-Epidithio-17α-oxahomoandrostane-3,17-dione</b>	<b>[M+Na]1+</b>	<b>-3.33</b>
			<b>389.14693</b>	<b>5337</b>	<b>389.14556</b>	<b>C9H10N2O3</b>	<b>4-Amino hippurate</b>	<b>[2M+H]1+</b>	<b>3.51</b>
			<b>389.17178</b>	<b>1901</b>	<b>389.17248</b>	<b>C20H30O5</b>	<b>PGD3</b>	<b>[M+K]1+</b>	<b>-1.81</b>
<b>2N=10~2N=20</b>	<b>391</b>	<b>Positive</b>	391.09958	1138	391.10011	C7H12O5	2-Isopropyl malate	[2M+K]1+	-1.35
			<b>391.17189</b>	<b>1055</b>	<b>391.17288</b>	<b>C8H16O4</b>	<b>6,8-Dihydroxy octanoate</b>	<b>[2M+K]1+</b>	<b>-2.53</b>
			<b>391.18751</b>	<b>1896</b>	<b>391.18813</b>	<b>C20H32O5</b>	<b>PGD2</b>	<b>[M+K]1+</b>	<b>-1.59</b>
			<b>391.22398</b>	<b>1441</b>	<b>391.22452</b>	<b>C21H36O4</b>	<b>Montanol</b>	<b>[M+K]1+</b>	<b>-1.38</b>

2N=10~2N=30	392	Positive	392.15354	1213	392.15513	C16H25NO10	Proacaciberin	[M+H] <sup>+</sup> 1+	-4.04
			392.29162	15526	392.29254	C22H43NO2	Anandamide (20:L, N-9)	[M+K] <sup>+</sup> 1+	-2.34
2N=10~2N=30	393.27	Positive	393.20338	1003	393.20378	C20H34O5	PGD1	[M+K] <sup>+</sup> 1+	-1.02
			393.23919	1133	393.24006	C19H37O6P	PA(16:0/0:0)[Cyclic]	[M+H] <sup>+</sup> 1+	-2.20
			393.27806	1130	393.27655	C22H42O3	2-Oxo docosanoate	[M+K] <sup>+</sup> 1+	3.83
E160F00h~E333F00h	395.18	Positive	395.07328	4137	395.07389	C16H20O9	1-O-Feruloyl-β-glucose	[M+K] <sup>+</sup> 1+	-1.55
E114F00h~E480F00h	401.27	Positive	401.25738	1016	-	-	Unknown	-	-
			401.26663	1378	401.26623	C23H38O4	2-Arachidonoyl glycerol	[M+Na] <sup>+</sup> 1+	0.99
E260F00h~E480F00h	413.27	Positive	413.08344	1442	413.08446	C16H22O10	Secologanate	[M+K] <sup>+</sup> 1+	-2.46
			413.09552	1628	413.09433	C8H14O2S2	Lipoate	[2M+H] <sup>+</sup> 1+	2.87
			413.12061	1390	413.12084	C17H26O9	7-Deoxy loganin	[M+K] <sup>+</sup> 1+	-0.56
			413.26581	1433	413.26623	C24H38O4	Apocholate	[M+Na] <sup>+</sup> 1+	-1.02
E129F00h~E480F00h	415.27	Positive	415.09928	1494	415.10011	C16H24O10	Loganate	[M+K] <sup>+</sup> 1+	-1.99
			415.13593	9134	415.13634	C10H12O4	5-Hydroxy coniferyl alcohol	[2M+Na] <sup>+</sup> 1+	-0.99
			415.17254	5231	415.17288	C9H16O4	Azelaic acid	[2M+K] <sup>+</sup> 1+	-0.82
			415.28170	1296	415.28188	C24H40O4	Deoxy cholate	[M+Na] <sup>+</sup> 1+	-0.44
E115F00h~E137F00h	424.18	Positive	424.28177	34209	424.28222	C25H39NO3	Myxalamid B	[M+Na] <sup>+</sup> 1+	-1.05
E129F00h~E160F00h	425.27	Positive	425.08424	1629	425.08446	C17H22O10	1-O-Sinapoyl-β-glucose	[M+K] <sup>+</sup> 1+	-0.51
			425.15663	6915	-	-	Unknown	-	-
2N=10~2N=20	425.9	Positive	426.16041	1677	426.16061	C16H27NO12	Hyaluronate	[M+H] <sup>+</sup> 1+	-0.46
			426.29810	718	426.29790	C20H44NO6P	PC(O-12:0/0:0)[U]	[M+H] <sup>+</sup> 1+	0.46
E114F00h~E480F00h	431.27	Positive	431.16709	13562	431.16603	C9H13NO5	Succinyl proline	[2M+H] <sup>+</sup> 1+	2.47
E114F00h~E480F00h	432.27	Positive	432.13474	1150	-	-	Unknown	-	-
			432.18749	1222	432.18938	C23H27N3O4	Benexate hydrochloride	[M+Na] <sup>+</sup> 1+	-4.37
2N=20~2N=30	437.27	Positive	437.15684	1291	437.15708	C23H26O7	Neoisostegane	[M+Na] <sup>+</sup> 1+	-0.54
			437.19329	1586	437.19346	C24H30O6	Estra-1,3,5(10)-triene-3,6,17-triol triacetate	[M+Na] <sup>+</sup> 1+	-0.39
			437.21040	1079	437.20887	C25H34O4	Pregna-5,16,20-triene-3β,20-diol diacetate	[M+K] <sup>+</sup> 1+	3.50
2N=10~2N=20	441.27	Positive	441.15187	2003	441.15199	C22H26O8	Syring aresinol	[M+Na] <sup>+</sup> 1+	-0.27
			441.33386	1172	441.33392	C27H46O3	7α,25-Dihydroxy cholesterol	[M+Na] <sup>+</sup> 1+	-0.13
2N=10~2N=20	442.9	Positive	443.07269	1195	443.07213	C10H7NO5	7,8-Dihydroxy kynurenate	[2M+H] <sup>+</sup> 1+	1.28
			443.20371	1203	443.20407	C18H35O10P	PG(6:0/6:0)	[M+H] <sup>+</sup> 1+	-0.80
			443.21909	1070	443.21943	C24H36O5	12-Hydroxy-3,6-dioxo-5-cholan-24-oate	[M+K] <sup>+</sup> 1+	-0.77
2N=20~2N=30	445.27	Positive	445.11024	1027	445.11067	C17H26O11	Ipolamiide	[M+K] <sup>+</sup> 1+	-0.98

E333F00h~E480F00h	449.18	Positive	445.14681	4940	445.14866	C15H28N2O11S	Desacetylmcythiol	[M+H] <sup>+</sup>	-4.16
			445.16403	1441	445.16365	C23H26N4O3	Argentine	[M+K] <sup>+</sup>	0.85
			445.18312	4901	445.18329	C22H30O8	Valtratum	[M+Na] <sup>+</sup>	-0.38
			445.23493	1053	445.23508	C24H38O5	3-Oxo cholate	[M+K] <sup>+</sup>	-0.34
E137F00h~E480F00h	451.18	Positive	449.10820	929	449.10784	C21H20O11	Kaempferol 7-O-glucoside	[M+H] <sup>+</sup>	0.80
			449.16789	1022	449.16669	C10H12N2O4	3-Hydroxy kynurenine	[2M+H] <sup>+</sup>	2.66
			451.04774	1462	451.04847	C7H10O7	Homocitrate	[2M+K] <sup>+</sup>	-1.62
			455.09356	1776	455.09487	C21H20O10	Apigenin 7-O-β-glucoside	[M+Na] <sup>+</sup>	-2.88
E114F00h~E115F00h	455.18	Positive	455.13104	1957	455.13126	C22H24O9	Medicarpin-3-O-glucoside	[M+Na] <sup>+</sup>	-0.47
			455.16782	1615	455.16602	C10H13NO5	L-Arogenate	[2M+H] <sup>+</sup>	3.94
			457.00882	1491	457.01098	C7H4N2O7	3,5-Dinitro salicylate	[2M+H] <sup>+</sup>	-4.73
			457.11049	1328	457.11052	C21H22O10	Naringenin 7-O-β-glucoside	[M+Na] <sup>+</sup>	-0.07
E114F00h~E480F00h	457.27	Positive	457.21019	1515	457.21159	C21H39O6P	cPA(18:1(11Z)/0:0)	[M+K] <sup>+</sup>	-3.06
			457.23486	1015	457.23332	C25H32N2O6	Vindoline	[M+H] <sup>+</sup>	3.38
			458.21318	1115	458.21465	C21H27N7O5	O-Demethyl puromycin	[M+H] <sup>+</sup>	-3.20
			459.06979	749	459.06881	C10H10O5	5-Hydroxy ferulate	[2M+K] <sup>+</sup>	2.14
2N=10~2N=30	458.18	Positive	459.12613	1520	459.12755	C17H23N4O9P	FMNH	[M+H] <sup>+</sup>	-3.08
			459.21397	1585	459.21435	C12H18O3	Jasmonate	[2M+K] <sup>+</sup>	-0.83
			460.85626	1265	-	-	Unknown	-	-
			461.17831	2252	461.18061	C24H28O9	6-Acetyl picropolin	[M+H] <sup>+</sup>	-4.99
E333F00h~E480F00h	459.27	Positive	463.06936	2619	-	-	Unknown	-	-
			463.20935	1080	463.20926	C23H36O7	Pravastatin	[M+K] <sup>+</sup>	0.19
			463.22452	1143	463.22586	C14H16N2	3,3'-Dimethyl benzidine	[2M+K] <sup>+</sup>	-2.88
			471.06798	840	471.06881	C21H20O10	Apigenin 7-O-β-glucoside	[M+K] <sup>+</sup>	-1.76
E114F00h~E480F00h	471.27	Positive	471.08987	1540	471.08979	C21H20O11	Kaempferol-3-glucoside	[M+Na] <sup>+</sup>	0.18
			473.10587	1665	473.10544	C21H22O11	Pentahydroxychalcone 4'-O-glucoside	[M+Na] <sup>+</sup>	0.92
			473.17822	4579	473.17567	C8H15N3O4	Acetyl citrulline	[2M+K] <sup>+</sup>	5.38
			473.19431	1062	473.19361	C24H34O7	Phorbol 13-butanoate	[M+K] <sup>+</sup>	1.47
E137F00h~E485F00h	485.27	Positive	485.14175	987	485.14197	C19H24N7O6	Tetrahydrofolate	[M+K] <sup>+</sup>	-0.45
			485.21509	1099	485.21459	C25H34O8	Hydrocortisone succinate	[M+Na] <sup>+</sup>	1.03
			485.30255	980	485.30277	C28H46O4	Di-N-Decyl phthalate	[M+K] <sup>+</sup>	-0.45
			485.36022	1649	485.36254	C31H48O4	1-Hydroxyvitamin D3 diacetate	[M+H] <sup>+</sup>	-4.77
E114F00h~E333F00h	489.9	Positive	489.95539	1514	-	-	Unknown	-	-

2N=10~2N=30	493.27	Positive	490.04523	1050	-	-	Unknown	-	-
			490.17718	1873	490.17726	C22H33N3O5S	Formylmethionyl-leucyl-phenylalanine methyl ester	[M+K]1+	-0.15
			493.13523	1316	493.13406	C23H24O12	Cicerin-7-O-glucoside	[M+H]1+	2.38
			493.18332	2337	493.18329	C26H30O8	Limonin	[M+Na]1+	0.06
2N=10~2N=20	499.18	Positive	493.20372	1167	493.20457	C20H38O11	Octyl β-1,6-galactofuranosyl-α-glucopyranoside	[M+K]1+	-1.73
			493.22002	1014	493.21983	C24H38O8	18-Acetoxy-PGF2α-11-acetate	[M+K]1+	0.39
			499.09780	1028	499.10011	C23H24O10	Afrormosin-7-O-glucoside	[M+K]1+	-4.62
			499.11474	2722	499.11632	C16H26N4O10S2	Bis-glutamyl cystine	[M+H]1+	-3.17
2N=10~2N=20	501.27	Positive	499.13487	1264	499.13649	C24H28O9	6-Acetyl picropolin	[M+K]1+	-3.25
			499.19366	1500	499.19386	C25H32O9	2-Methoxyestrone 3-glucuronide	[M+Na]1+	-0.39
			499.23090	1122	499.23024	C26H36O8	Retinoyl β-glucuronide	[M+Na]1+	1.32
			501.13774	1043	501.13674	C23H26O11	Homoferreirin-7-O-glucoside	[M+Na]1+	2.00
E115F00h~E333F00h	503.36	Positive	501.17347	1295	501.17327	C21H34O11	Patrinoside	[M+K]1+	0.39
			503.22510	1056	503.22756	C27H34O9	Verrucaric A	[M+H]1+	-4.89
			503.26122	1290	503.25919	C27H44O4S	7-Dehydrocholesterol-3-sulfate ester	[M+K]1+	4.03
			503.33357	2103	503.33431	C28H48O6	Brassinolide	[M+Na]1+	-1.47
2N=20~2N=30	512.27	Positive	512.05016	1474	-	-	Unknown	-	-
			512.14597	2082	-	-	Unknown	-	-
2N=10~2N=20	519.27	Positive	519.18399	1326	519.18609	C26H30O11	Simplexoside	[M+H]1+	-4.05
			519.22025	1121	-	-	Unknown	-	-
			519.32940	1079	-	-	Unknown	-	-
2N=10~2N=20	527.27	Positive	527.15841	1634	527.15826	C18H32O16	Raffinose	[M+Na]1+	0.28
			531.09036	2328	531.08994	C23H24O12	Cicerin-7-O-glucoside	[M+K]1+	0.79
			531.18381	923	531.18369	C25H32O11	Gibberellin 2-O-β-glucoside	[M+Na]1+	0.23
E176F00h~E484F00h	533.36	Positive	531.25680	1583	531.25886	C29H38O9	Uscharidin	[M+H]1+	-3.88
			533.20008	1185	533.19934	C25H34O11	Gibberellin 2-O-β-glucoside	[M+Na]1+	1.40
			533.27272	1282	533.27451	C29H40O9	Calactin	[M+H]1+	-3.36
			533.34509	1190	533.34728	C31H48O7	1α,25-Dihydroxy-22-oxacholecalciferol 3-hemiglutarate	[M+H]1+	-4.11
E137F00h~E160F00h	543.27	Positive	543.13296	18401	543.13220	C18H32O16	Raffinose	[M+K]1+	1.40
E137F00h~E160F00h	544.27	Positive	544.13612	3009	-	-	13C Isotope of Raffinose	[M+K]1+	-
E114F00h~E115F00h	545.27	Positive	545.13012	1317	-	-	41K Isotope of Raffinose	[M+K]1+	-
E137F00h~E160F00h	553.36	Positive	553.05768	1382	553.05655	C6H13O10P	6-Phospho gluconate	[2M+H]1+	2.04
			553.27812	10018	-	-	Unknown	-	-



E129F00h~E162F00h	556	Positive	556.28020	1889	556.28000	C26H48NO7P	PC(18:3(9Z,12Z,15Z)/0:0)	[M+K]1+	0.36
E114F00h~E162F00h	557.36	Positive	557.12101	2379	-	-	Unknown	-	-
			557.20039	1575	-	-	Unknown	-	-
			557.25217	1361	557.25338	C34H36O7	Ingenol 3,20-Dibenzoate	[M+H]1+	-2.17
2N=10~2N=30	561.17	Positive	561.20986	1623	561.20966	C27H38O10	Trilobolide	[M+K]1+	0.36
			561.30363	1179	561.30356	C26H50O10	Tween 20	[M+K]1+	0.13
E115F00h~E137F00h	563.27	Positive	563.09567	1243	563.09487	C15H10O5	Apigenin	[2M+Na]1+	1.42
			563.22579	1237	563.22665	C14H18N2O3	Methohexital	[2M+K]1+	-1.52
			563.24663	1130	563.24869	C29H38O11	Eriocarpin	[M+H]1+	-3.66
E333F00h~E484F00h	566.09	Positive	565.99281	2705	-	-	Unknown	-	-
			566.08931	1360	566.09114	C17H27N3O13P2	dTDP-4-oxovancosamine	[M+Na]1+	-3.23
E114F00h~E484F00h	573.36	Positive	573.10094	1009	573.10276	C15H10O6	Kaempferol	[2M+H]1+	-3.17
			573.19508	1150	573.19264	C12H16NO7	N-Glucosyl nicotinate	[2M+H]1+	4.27
			573.28438	1548	573.28305	C11H26NO4P	Glycerol phosphoryl choline	[2M+K]1+	2.31
			573.41272	1278	573.41287	C29H61NO6P	PC(O-18:0/O-3:1(2E))[U]	[M+Na]1+	-0.27
E333F00h~E480F00h	587.36	Positive	587.13894	1087	587.13713	C26H28O14	Apigenin 7-O-[β-Apiosyl-(1->2)-β-Glucoside]	[M+Na]1+	3.08
2N=10~2N=20	589.36	Positive	589.22647	6142	589.22796	C15H18O6	Tutin	[2M+H]1+	-2.52
2N=10~2N=30	591.45	Positive	591.01295	863	591.01244	C6H13O10P	6-Phospho gluconate	[2M+K]1+	0.87
			591.22465	817	591.22206	C7H16N8O4	Trimethylene tetra urea	[2M+K]1+	4.38
			591.27777	1120	591.27524	C30H48O7S	Tri terpenoid	[M+K]1+	4.28
2N=10~2N=20	603.27	Positive	603.11118	4393	603.11151	C14H11N3O3S	Apigenin 7-O-[β-Apiosyl-(1->2)-β-Glucoside]	[M+K]1+	-0.54
2N=20~2N=30	613.63	Positive	613.48268	2563	613.48265	C39H64O5	DG(18:3(6Z,9Z,12Z)/18:3(6Z,9Z,12Z)/0:0)	[M+H]1+	0.05
E162F00h~E485F00h	615.36	Positive	615.40255	2135	615.39963	C31H61O8P	PA(14:0/14:0)	[M+Na]1+	4.75
2N=20~2N=30	629.45	Positive	629.45284	1791	629.45418	C37H66O5	DG(16:1(9Z)/18:2(9Z,12Z)/0:0)	[M+K]1+	-2.13
E114F00h~E260F00h	633.36	Positive	633.11995	1190	633.12163	C27H30O15	Kaempferol 3-O-rhamnoside-7-O-glucoside	[M+K]1+	-2.66
			633.48542	884	633.48548	C37H70O5	DG(18:0/16:1(9Z)/0:0)	[M+K]1+	-0.10
2N=20~2N=30	635.54	Positive	635.41274	1437	635.41536	C36H58O9	Hederagenin-3-o-glucoside	[M+H]1+	-4.13
			635.47635	1476	635.47824	C20H31NO2	17β-Hydroxy-4,17-dimethyl-4-azaandrost-5-en-3-one	[2M+H]1+	-2.97
			635.50124	1203	635.50113	C37H72O5	DG(17:0/0:0/17:0)-D5	[M+K]1+	0.17
2N=10~2N=20	637.36	Positive	637.18891	7126	637.18994	C10H22NO7P	PC(0:0/2:0)[U]	[2M+K]1+	-1.62
E114F00h~E115F00h	644.8	Positive	644.67270	1633	-	-	Unknown	-	-
			645.19781	1101	645.19976	C36H34N2O7	Cancentrine	[M+K]1+	-3.03
E260F00h~E480F00h	671.36	Positive	671.36796	1116	671.37085	C20H28O3	15-PGA2	[2M+K]1+	-4.30

			671.40577	970	671.40487	C34H65O8P	PA(17:0/14:1(9Z))	[M+K]1+	1.34
E114F00h~E480F00h	685.45	Positive	685.36411	1127	685.36268	C20H25N3O	Lysergate diethylamide	[2M+K]1+	2.08
E114F00h~E137F00h	722.9	Positive	723.19423	1795	723.19559	C12H22O11	Sucrose	[2M+K]1+	-1.88
E114F00h~E160F00h	745.63	Positive	745.62191	1147	745.62192	C22H43NO2	Anandamide (20:L, N-9)	[2M+K]1+	-0.01
2N=20~2N=30	784.63	Positive	784.58476	1515	784.58508	C44H82NO8P	PC(16:0/20:3(11E,14E,17E))[U]	[M+H]1+	-0.41
2N=10~2N=20	793.36	Positive	793.43590	744	793.43689	C42H64O14	Gypsogenin 3-O-rhamnosyl glucuronide	[M+H]1+	-1.24
			793.52235	1162	793.52266	C43H78O10	1-18:2-2-16:0-Monogalactosyl diacylglycerol	[M+K]1+	-0.39
E114F00h~E480F00h	794.54	Positive	794.50936	6723	794.50967	C42H78NO8P	PC(16:0/18:3(5E,9Z,12Z))[U]	[M+K]1+	-0.38
2N=10~2N=20	796.63	Positive	796.52557	7609	796.52532	C42H80NO8P	PC(16:0/18:2(2Z,4Z))	[M+K]1+	0.32
			796.60055	1475	796.59809	C44H88NO6P	PC(O-18:1(9Z)/O-18:1(9Z))	[M+K]1+	3.09
2N=10~2N=20	797.72	Positive	797.51793	14542	797.51742	C45H74O10	1,2 Di-(9Z,12Z,15Z-octadecatrienoyl)-3-O-β-galactosyl-sn-glycerol	[M+Na]1+	0.64
E114F00h~E162F00h	798.63	Positive	798.52029	7287	798.51984	C45H78NO6P	1-(8-[3]-Ladderane-octanyl)-2-(8-[3]-ladderane-octanyl)-sn-glycerophosphoethanolamine	[M+K]1+	0.57
E115F00h~E137F00h	815.54	Positive	815.48995	14486	815.48588	C24H36O4	3α-Hydroxy-12-oxo-5β-chol-9(11)-en-24-oate	[2M+K]1+	4.99
			815.49799	10883	815.49498	C41H76O12S	SQDG(16:0/16:1(11Z))	[M+Na]1+	3.70
E114F00h~E137F00h	816.54	Positive	816.49101	8132	816.49402	C44H76NO8P	PC(18:3(8E,10E,12E)/18:3(8E,10E,12E))[U]	[M+K]1+	-3.68
2N=10~2N=30	817.54	Positive	817.52373	3413	817.52266	C45H78O10	1-18:1-2-18:3-Monogalactosyl diacylglycerol	[M+K]1+	1.31
E137F00h~E162F00h	818.54	Positive	818.50962	4272	818.50967	C44H78NO8P	PC(16:1(9Z)/20:4(5Z,8Z,11Z,14Z)/0:0)	[M+K]1+	-0.05
E162F00h~E333F00h	819.54	Positive	819.39675	1159	819.39277	C41H64O14	Digoxin	[M+K]1+	4.86
			819.43014	1189	819.43380	C39H74O11P2	PPA(18:1(9Z)/18:1(9Z))	[M+K]1+	-4.47
			819.51147	2074	819.51466	C44H77O10P	PG(18:1(11Z)/20:4(5Z,8Z,11Z,14Z))	[M+Na]1+	-3.89
			819.53889	1016	819.53831	C45H80O10	1-18:1-2-18:2-Monogalactosyl diacylglycerol	[M+K]1+	0.71
E115F00h~E485F00h	820.54	Positive	820.52421	3772	820.52532	C44H80NO8P	PC(18:2(2E,4E)/18:2(2E,4E))	[M+K]1+	-1.35
2N=10~2N=20	821.54	Positive	821.52621	2110	821.53031	C44H79O10P	PG(18:0/20:4(5Z,8Z,11Z,14Z))	[M+Na]1+	-4.99
2N=10~2N=30	822.54	Positive	822.54088	1697	822.54097	C44H82NO8P	PC(18:0/18:3(9Z,12Z,15Z)/0:0)	[M+K]1+	-0.10
2N=10~2N=30	823.54	Positive	823.54340	1109	823.54596	C44H81O10P	PG(18:0/20:3(5Z,8Z,11Z))	[M+Na]1+	-3.11
2N=10~2N=30	828.54	Positive	828.47400	3812	828.47400	C42H69NO15	Leucomycin A3	[M+H]1+	0.00
			828.50795	1150	-	-	Unknown	-	-
E137F00h~E160F00h	829.54	Positive	829.48662	5045	829.48627	C45H74O11	1-18:3-2-18:3-Monogalactosyl diacylglycerol	[M+K]1+	0.42
E137F00h~E480F00h	861.54	Positive	861.47599	3282	861.47836	C25H34O6	21-Hydroxypregn-4-ene-3,20-dione hydrogen succinate	[2M+H]1+	-2.75
2N=10~2N=20	937.63	Positive	937.58463	924	937.58830	C51H84O15	1-18:3-2-18:3-Digalactosyl diacylglycerol	[M+H]1+	-3.92
2N=10~2N=30	941.54	Positive	941.35461	843	941.35686	C17H30N2O13	Galactosyl-3-(N-acetyl-β-galactosaminy)-serine	[2M+H]1+	-2.39
			941.54941	1440	-	-	Unknown	-	-

2N=10~2N=30	953.63	Positive	953.56050	4967	953.55983	C49H86O15	1-16:0-2-18:3-Digalactosyl diacylglycerol	[M+K]1+	0.70
2N=10~2N=20	959.63	Positive	959.56943	1765	959.57025	C51H84O15	1-18:3-2-18:3-Digalactosyl diacylglycerol	[M+Na]1+	-0.85
E129F00h~E137F00h	975.63	Positive	975.54470	11590	975.54418	C51H84O15	1-18:3-2-18:3-Digalactosyl diacylglycerol	[M+K]1+	0.53

**Table S10.** List of annotated  $m/z$  signals where signal identities were confirmed by comparing MS<sup>n</sup> fragmentation pattern with that of standard/s

$m/z$	Ionization mode	Accurate $m/z$	Max. Intensity	Theoretical $m/z$	Molecular Formula	Identity	Adduct	PPM Error	MSI* Level of identification
116.09	Negative	116.07155	1962	116.07170	C5H11NO2	Valine	[M-H]1-	-1.31	1
118.09	Negative	118.05079	19614	118.05097	C4H9NO3	Threonine	[M-H]1-	-1.50	1
133.09	Negative	133.01404	689515	133.01425	C4H6O5	Malate	[M-H]1-	-1.56	1
135	Negative	135.02970	55697	135.02990	C4H8O5	Threonate	[M-H]1-	-1.47	1
147	Negative	147.04507	511	147.04515	C9H8O2	Cinnamate	[M-H]1-	-0.56	1
163	Negative	163.03983	29190	163.04007	C9H8O3	<i>p</i> -Coumarate	[M-H]1-	-1.46	1
179.09	Negative	179.03491	2291	179.03498	C9H8O4	Caffeate	[M-H]1-	-0.41	1
	Negative	179.05589	2268	179.05611	C6H12O6	Glucose	[M-H]1-	-1.25	1
185.09	Negative	184.98556	21029	184.98567	C3H7O7P	3-Phospho glycerate	[M-H]1-	-0.57	1
191.09	Negative	191.05585	164745	191.05611	C7H12O6	Quinate	[M-H]1-	-1.38	1
193.09	Negative	193.05061	13417	193.05063	C10H10O4	Ferulate	[M-H]1-	-0.12	1
259.18	Negative	259.02271	24036	259.02245	C6H13O9P	Glucose 6-phosphate	[M-H]1-	1.02	1
341.27	Negative	341.10886	210747	341.10894	C12H22O11	Sucrose	[M-H]1-	-0.23	1
377.27	Negative	377.08556	36887	377.08562	C12H22O11	Sucrose	[M+Cl]1-	-0.15	1
503.27	Negative	503.15904	1878	503.16176	C18H32O16	Raffinose	[M-H]1-	-5.41	1
539.27	Negative	539.13550	2797	539.13844	C18H32O16	Raffinose	[M+Cl]1-	-5.45	1
116	Positive	116.07030	9205	116.07061	C5H9NO2	Proline	[M+H]1+	-2.64	1
118.09	Positive	118.08587	1468640	118.08626	C5H11NO2	Both Valine and Betaine	[M+H]1+	-3.27	1
120.09	Positive	120.06515	37635	120.06552	C4H9NO3	Threonine	[M+H]1+	-3.09	1
132	Positive	132.10146	21097	132.10191	C6H13NO2	Leucine	[M+H]1+	-3.38	1
139.9	Positive	140.06789	56514	140.06820	C5H11NO2	Betaine	[M+Na]1+	-2.21	1
147.09	Positive	147.11243	3326	147.11281	C6H14N2O2	Lysine	[M+H]1+	-2.55	1
182	Positive	182.08037	1542	182.08117	C9H11NO3	Tyrosine	[M+H]1+	-4.40	1
203.18	Positive	203.05182	2016	203.05261	C6H12O6	Glucose	[M+Na]1+	-3.90	1
219.09	Positive	219.02584	46171	219.02655	C6H12O6	Glucose	[M+K]1+	-3.23	1
365.27	Positive	365.10457	21308	365.10544	C12H22O11	Sucrose	[M+Na]1+	-2.37	1
381.18	Positive	381.07882	821755	381.07937	C12H22O11	Sucrose	[M+K]1+	-1.45	1
527.27	Positive	527.15841	1634	527.15826	C18H32O16	Raffinose	[M+Na]1+	0.28	1
543.27	Positive	543.13296	18401	543.13220	C18H32O16	Raffinose	[M+K]1+	1.40	1

\* Metabolomics Standards Initiative

**Table S11.** Contribution of metabolomic variables to the first canonical discriminant functions obtained for the studied ecotypes of *B. distachyon* (5 ecotypes, 60 replicates), *B. stacei* (3, 36) and *B. hybridum* (4, 48). Contribution values of positive and negative metabolites to the functions. (a) Metabolites not included in the discriminant analysis (see Figure 7). (b) Duplicate *m/z* signals with same metabolite annotations not included in the discriminant analysis (see Figure S1). \*  $p < 0.05$ .

Positives	Function		Negatives	Function	
	1	2		1	2
p556(a)	0.17135729 *	-0.03268181	n487.18(a)	0.10691284 *	0.05916933
p745.63(a)(b)	-0.11589141 *	0.06378592	n683.27(a)(b)	-0.10284352 *	-0.00752735
p821.54(a)	0.07819518 *	0.04588131	n534.09(a)	0.0909125 *	0.00448834
p644.8(a)	0.06599859 *	0.0324704	n449.09(a)(b)	0.08101284 *	-0.024566
p784.63(a)	0.06406575 *	0.0068279	n493.18(a)	-0.07917416 *	-0.05798212
p797.72(a)	-0.055852 *	-0.03887777	n773.36(a)	-0.07093755 *	0.06496141
p722.9(a)(b)	0.05001288 *	-0.01978989	n281.18	0.066872 *	-0.04709359
p793.36(a)	-0.04841338 *	-0.03791324	n541.27(a)(b)	-0.064112 *	-0.02159938
p531.17(a)	0.04745779 *	0.00035793	n711.45(a)(b)	0.06403728 *	-0.04741422
p563.27(a)	0.04705143 *	-0.01739839	n91.09(a)(b)	-0.06388151 *	0.04288624
p798.63(a)	-0.0424412 *	0.01930392	n564.27(a)(b)	-0.06056355 *	0.01539439
p671.36(a)	0.04182909 *	0.01524383	n385.18	-0.05910667 *	0.0413003
p185.09	0.03668609 *	0.00667615	n346.09	0.05868734 *	0.05402804
p74.09(a)	0.03543903 *	0.00283447	n420.18(a)	0.05716986 *	0.04242448
p161	-0.03533044 *	-0.00699052	n315.18	0.0570832 *	0.03625291
p829.54(a)	-0.03271584 *	0.02505552	n637.09(a)	0.05337797 *	0.04719185
p543.27	-0.03113857 *	-0.00918161	n831.63(a)	-0.0528396 *	-0.01228922
p425.27	-0.03018125 *	-0.0175534	n319.18	0.05219099 *	-0.01174737
p214.09	0.02912067 *	-0.00656418	n588.09(a)	0.05087734 *	-0.0392621
p822.54(a)	-0.02784804 *	-0.00830355	n183.09	-0.04969392 *	0.02863842

p959.63(a)	0.02773534 *	-0.02354728	n447.18(a)	0.04752689 *	0.01239004
p208.09	-0.02742153 *	-0.01300387	n567.17(a)(b)	0.04642749 *	-0.0412682
p259	0.02669739 *	-0.01271277	n237.09	0.04425317 *	-0.02893843
p375.18	-0.02651562 *	-0.02022726	n208.18	0.04419089 *	-0.02435153
p389.18	-0.026197 *	-0.00441676	n421.18(a)	0.04409848 *	0.04333511
p371.27	-0.02599031 *	-0.01742533	n364.18	-0.04384556 *	0.01160201
p251.09	0.02595212 *	-0.00436497	n236.09	0.04271356 *	0.02042987
p387.27	-0.02591783 *	-0.0170768	n168	0.04159374 *	0.01714706
p561.17	0.02540061 *	0.00341965	n837.63(a)	-0.04071231 *	0.0295045
p373.27	-0.02532623 *	-0.01741389	n218.09	0.0400986 *	0.01422365
p415.27	-0.02515384 *	-0.00858066	n367.18	-0.0381501 *	-0.01018694
p473.27	-0.02362285 *	-0.01995903	n397.09	0.03445392 *	0.01786764
p431.27	-0.02362083 *	-0.01891414	n431.18(a)	0.03408869 *	0.01622681
p144	-0.02319491 *	0.01195547	n563.27(a)(b)	-0.03300724 *	0.00163231
p275.08	0.02311835 *	-0.02001975	n323.18	0.02943576 *	0.00288512
p187.09(b)	0.02294846 *	-0.00524348	n241.09	0.02812934 *	0.00749078
p249.09	0.02291883 *	-0.00866178	n398.18	0.02631795 *	0.01309024
p455.18	-0.02260658 *	-0.02157215	n239.09(b)	0.0257073 *	-0.02437268
p401.27	-0.02245926 *	-0.01985136	n593.27(a)	0.02464305 *	0.01379899
p194.09	-0.02229803 *	-0.02123977	n269.18	0.02383524 *	-0.01365108
p461.27	-0.0217677 *	-0.01672509	n371.27	0.02367175 *	0.00699701
p432.27	-0.0215659 *	-0.01536629	n741.63(a)(b)	-0.02320561 *	0.01094665
p374.18	-0.02134043 *	-0.01742896	n158	-0.02273181 *	0.00217017
p445.27	-0.02066277 *	0.01472831	n355.18	0.02147256 *	0.0014226
p861.54(a)	-0.02036182 *	-0.01064722	n124	-0.02140078 *	-0.00086583
p545.27(b)	-0.02029911 *	0.01865338	n198	0.01735611 *	-0.01100114
p316.18	0.01997464 *	-0.00893347	n745.63(a)	0.01603167 *	-0.00187849
p413.27	-0.01991215 *	-0.00873967	n182.09	-0.01414265 *	0.01003864
p941.54(a)	0.0198647 *	0.00091987	n488.18(a)	-0.01353696 *	-0.0044062
p245.09	0.01907059 *	0.0042518	n419.09(a)	-0.01145159 *	0.00603576

p458.18	-0.01865699 *	-0.00532671	n311.18	-0.010112 *	0.00234456
p459.27	-0.01843039 *	-0.01662293	n440.18(a)	-0.00666115 *	0.00534058
p287.18	-0.01809892 *	-0.01514769	n429.18(a)	-0.00282702 *	-0.00188081
p107	-0.01662211 *	0.01444534	n640.9(a)	-0.0336556	-0.1509666
p485.27	-0.0165864 *	0.01135197	n463.18(a)	0.0215549	0.12164412 *
p147.09	0.01641064 *	-0.00786311	n455.18(a)	-0.01266939	0.12128073 *
p451.18(b)	0.01570822 *	0.00531923	n503.27(a)(b)	0.01092592	0.11386974 *
p314	0.01533471 *	0.00853629	n187.09	0.02342785	0.11365859 *
p263.08	0.01425238 *	-0.00827436	n163	0.03210545	-0.11349963 *
p343.18	-0.01417365 *	0.00059248	n191.09	0.03388844	0.10900074 *
p794.54	0.01383347 *	0.00874327	n174.09(b)	0.030881	0.10850088 *
p573.36(a)	0.01372934 *	0.01212334	n408.09(a)	0.00018872	-0.10739704 *
p246.09	0.01318767 *	-0.00346026	n203.09	0.00485209	0.10735808 *
p188.09	-0.01292403 *	-0.00731651	n103.09	0.0393604	0.10704601 *
p225	-0.01176425 *	0.00153209	n192.09(b)	0.03358449	0.10032185 *
p271.08	0.01165346 *	-0.009562	n116.09(b)	0.02953307	0.10000319 *
p236.09	-0.01144151 *	0.00825975	n173.09	0.02776066	0.09764381 *
p393.27	0.00963716 *	-0.00780288	n565.17(a)	-0.06795524	0.09750186 *
p227.09	0.00875656 *	-0.00325636	n204.09	0.00849998	0.09742876 *
p102	-0.00842455 *	-0.00764412	n118.09(b)	0.01923052	0.09674183 *
p319.18	0.00734949 *	-0.00575643	n407.18(a)	-0.05482895	0.09624705 *
p953.63(a)	0.0032139 *	0.0002241 *	n425.18(a)	-0.02807029	0.09499261 *
p591.45(a)	0.05910707	-0.12273571 *	n179.09	-0.01418672	-0.09447149 *
p828.54(a)	-0.00735892	0.09665576 *	n540.17(a)	-0.0377537	-0.09166627 *
p818.54(a)	-0.01863799	0.0940137 *	n379.18	-0.00422326	0.09144565 *
p296.18	0.01865014	0.08953956 *	n289.18	0.02979477	0.08864315 *
p823.54(a)	0.00785513	0.0876995 *	n135	0.03229688	0.08707913 *
p341.9	0.00718879	0.0805188 *	n89.09(a)	-0.04618635	0.08589293 *
p241	0.00780712	0.07898506 *	n341.27(b)	0.0022882	0.08534252 *
p587.36(a)(b)	-0.02470468	-0.07816338 *	n126	0.02603927	0.08445796 *

p139.9	-60.0996E-05	0.07639693 *	n259.18	0.00841547	0.08351186 *
p615.36(a)	-0.05184503	0.07278678 *	n221.09	-0.01897209	0.08203813 *
p685.45(a)	0.01842174	0.07173456 *	n223.09	0.03117499	0.08197337 *
p613.63(a)	-0.03080696	0.07123063 *	n277.18	0.0197725	0.08196764 *
p118.09	-0.0211047	0.0692034 *	n515.17(a)	0.07881158	0.08099477 *
p235.09	-0.01176515	0.06700429 *	n72.09(a)	0.04151713	0.08085707 *
p141.9	0.00867468	0.06663724 *	n207.09	0.00443291	0.08054853 *
p603.27(a)(b)	-0.01252154	-0.06611056 *	n623.17(a)	0.03909908	-0.07972835 *
p243	0.00653912	0.06572586 *	n342.18(b)	0.00505333	0.07896249 *
p796.63(a)	0.01780884	0.06554277 *	n142	0.01873417	0.07854977 *
p119.09	-0.01855705	0.06426201 *	n129.09	-0.02048275	0.0782987 *
p442.9	-0.00707965	0.05958822 *	n117	-0.00036284	0.07786907 *
p156(b)	-0.01589888	0.05883721 *	n145.09	0.03181791	0.07785151 *
p157(b)	-0.01271255	0.05842928 *	n437.18(a)	-0.02854968	0.0768511 *
p589.36(a)	-0.0212538	-0.05818232 *	n134.09(b)	0.03747538	0.07650159 *
p637.36(a)	-0.02656575	0.05755332 *	n119	-0.0045675	0.07605684 *
p158(b)	-0.01111817	0.0566398 *	n193.09	0.01936176	0.07591462 *
p975.63(a)	0.02493056	0.0564019 *	n133.09	0.03943679	0.07582015 *
p257.08	-0.01157654	0.05552115 *	n195.18	-0.03599773	0.075591 *
p268.18	0.01605075	-0.05157071 *	n387.18	-0.01758294	0.0754981 *
p303.18	-0.04913525	-0.04941833 *	n144.09	0.02278051	0.07467059 *
p635.54(a)	0.00127512	-0.04657567 *	n271	0.01878765	0.07448961 *
p359.18	0.0179383	0.04466575 *	n232.09	0.01972189	0.07391333 *
p138.09	0.0062768	-0.04399293 *	n229.09	0.02019992	0.07377056 *
p544.27(a)(b)	-0.01502589	-0.04176155 *	n413.18(a)	-0.00102123	0.07279405 *
p381.18	0.00667761	-0.04136004 *	n359.18(b)	0.01672865	0.0718037 *
p244.18	-0.00715239	0.04129091 *	n194.09	0.02392555	0.07154963 *
p817.54(a)	-0.02658129	0.0408488 *	n222.09	0.02347324	0.07150948 *
p320.18	-0.00615287	0.03973365 *	n377.27(b)	-0.00581768	0.07146513 *
p820.54(a)	-0.01623826	0.03973056 *	n216.09	0.00763139	0.0710507 *



p98(a)	-0.03101881	-0.03880071 *	n217.18(b)	0.00162752	0.07099585 *
p123	-0.01395276	0.03835744 *	n343.18	-0.00260522	0.07036414 *
p269.18	0.01104821	-0.03828728 *	n327.18	0.01990253	0.06989065 *
p557.36(a)	-0.00126497	0.0379244 *	n104.09(b)	-0.01102211	0.06920225 *
p351.18	0.00078614	-0.03753876 *	n185.09	0.02483926	0.06861393 *
p383.18(b)	0.00400772	-0.03723309 *	n213.09	0.02349962	0.06846964 *
p463.27	-0.01240458	-0.0372161 *	n382.18	0.02375535	-0.06828923 *
p382.18(b)	0.00653646	-0.03640474 *	n380.18	-0.02310335	0.06820798 *
p355.18(b)	-0.01762868	-0.03632206 *	n231.09	0.02470378	0.06794703 *
p527.27(b)	-0.03039452	-0.03588899 *	n123	-0.00142824	0.06726252 *
p326	-0.00401082	0.03583792 *	n329.18	0.02361396	0.06709435 *
p365.27(b)	-0.01881622	-0.03533682 *	n479.18(a)	-0.03837487	0.06706999 *
p315	-0.00131623	0.03406258 *	n247.09	0.02274443	-0.06677487 *
p217.18	-0.00414669	-0.03386248 *	n475.09	0.00908495	0.06642628 *
p501.27	-0.02081549	-0.03304894 *	n210.09	0.002316	-0.06623342 *
p123.9	-0.02159087	0.03191294 *	n101.09	0.00838092	0.06576783 *
p425.9	-0.00166877	0.03190432 *	n309.18	0.0123491	0.06500331 *
p384.18	-0.00528392	-0.03164316 *	n245.09	0.01856076	0.06497199 *
p299.18	-0.00798639	-0.03102332 *	n151	-0.01498735	0.06256087 *
p815.54(a)	-0.0205414	0.03063717 *	n535.27(a)	-0.03615952	-0.0623386 *
p441.27	-0.01681731	-0.03037066 *	n815.72(a)	-0.0496955	0.06158677 *
p141	-0.02024014	0.03025257 *	n121	-0.04116031	0.06071729 *
p111.54	-0.01773672	0.03021229 *	n211.09	0.00406941	-0.0600454 *
p307.18	-0.00060585	-0.02984555 *	n201.09	0.00186953	0.05986275 *
p323.18	-0.00786287	-0.02958797 *	n279.18	0.00486933	0.0597708 *
p182	-0.0022156	0.02949432 *	n212.09	0.00408485	-0.05970942 *
p283.18	-0.01594478	-0.02929479 *	n566.27(a)	-0.02671568	-0.05964453 *
p512.27	-0.0043626	-0.02857765 *	n423.18(a)	-0.00825993	0.05879851 *
p367.18	0.00487315	-0.02845452 *	n278.18	0.01254602	0.05874761 *
p215	0.00801344	-0.02838471 *	n389.18	0.00929836	0.05815306 *

p385.18	-0.02151347	-0.02837774 *	n71.09(a)	0.05569231	0.05809948 *
p489.9	-0.00622776	0.02775259 *	n459.09(a)	-0.01246579	0.05746197 *
p377.27(b)	0.00164105	-0.02763185 *	n107.09	-0.02446038	0.05741966 *
p424.18	0.00027382	-0.02754791 *	n253.09	0.02649381	0.05708799 *
p503.36	-0.0179622	-0.02715203 *	n266.08	0.02004362	-0.05698637 *
p366.27(b)	-0.02445893	-0.02660359 *	n388.18	0.00748036	0.05653227 *
p437.27	0.00245714	-0.02649755 *	n298.18	0.00776899	0.05627399 *
p116	-0.00944816	0.02562608 *	n105.09	0.03843572	0.05584997 *
p120.09	-0.01469729	0.02543706 *	n316.18	0.04461231	0.05425913 *
p345.18	-0.02143302	-0.0254367 *	n597.17(a)	-0.02596052	0.0540724 *
p219.09	0.00392677	-0.02539782 *	n513.17(a)(b)	0.01067602	0.05390167 *
p229.18	-0.01409067	-0.02511075 *	n152.09	-0.01139991	-0.05373961 *
p127.09	0.00778045	0.02475968 *	n297.18	0.0063085	0.05359655 *
p132.44(b)	-0.01382071	0.02462501 *	n215.09(b)	-0.00627284	0.05358247 *
p139	-0.00179733	-0.02444608 *	n228.09	-0.00083312	0.05351008 *
p132	-0.01266488	0.02428972 *	n471.18(a)	-0.00793498	0.05288463 *
p286.08(b)	0.01700757	0.02408596 *	n793.72(a)	-0.03184692	0.05280508 *
p150	-0.00339609	0.0240551 *	n435.18	0.05096564	-0.05271241 *
p533.36	0.00606747	-0.02359665 *	n393.09	0.02536316	0.05269626 *
p392	0.0059922	-0.02327506 *	n197.18	-0.01551853	0.05124483 *
p471.27	-0.01911026	-0.02309861 *	n87.09(a)	0.02000473	0.0506425 *
p553.36(a)	0.01140363	-0.02276638 *	n147	-0.0175082	-0.05036831 *
p357.27	-0.02044951	-0.02271193 *	n369.09	-0.00618641	0.04965657 *
p261.18	-0.01258507	-0.0227078 *	n344.09	0.03273682	-0.04940203 *
p629.45	0.00138415	0.02253766 *	n347.09	0.04195793	0.04922704 *
p279.18	0.01199126	-0.02234593 *	n340.09	0.03897618	-0.04916661 *
p457.27	-0.02158574	-0.02215635 *	n88.09(a)	0.01755829	0.04907508 *
p499.18	-0.01646321	-0.02208113 *	n303.18	0.03263171	0.04892497 *
p294.18	-0.01401933	-0.02168967 *	n274.08	0.04087023	0.04860858 *
p149.54	-0.00710405	0.02159323 *	n136	0.03812048	-0.04851476 *

p282.18	-0.00743525	-0.02157041 *	n181.09	0.04275054	0.04783606 *
p816.54(a)	0.00055569	0.0214223 *	n362.18	0.01801329	0.04738684 *
p264.18(b)	-0.01390805	-0.02115437 *	n453.18(a)	-0.00567966	0.04698278 *
p295.18	-0.01017743	-0.02107792 *	n143.09	-0.01264473	0.0468813 *
p173	-0.0088152	-0.02082232 *	n171	0.02081014	0.04534125 *
p128	0.0068011	0.02076778 *	n251.18	0.04387136	0.04490878 *
p62.09(a)	0.00341026	-0.02065367 *	n184.09	-0.01123123	0.04467933 *
p364.09	-0.0037785	0.02061619 *	n224.09(b)	0.03869826	0.04406147 *
p176	0.01526482	-0.02039539 *	n132.09	-0.02962011	0.04342782 *
p203.18(b)	-0.01547632	-0.02029161 *	n521(a)(b)	60.2714E-05	-0.04162318 *
p184	-0.00493959	0.0201031 *	n257.08	-0.00248912	0.04104685 *
p233.18	-0.01066595	-0.01998763 *	n255.27	-0.00951153	0.0402138 *
p84.09(a)	0.00306756	0.01997187 *	n295.18	0.00651354	0.03989269 *
p224.09	-0.00816055	-0.01959942 *	n819.63(a)	0.0169355	0.03972643 *
p278.18(b)	0.0154575	-0.01930246 *	n743.63(a)	-0.0045642	0.03957746 *
p191	-0.00630511	0.01883918 *	n469.18(a)(b)	-0.00269068	0.03851735 *
p159	-0.00532332	0.01863778 *	n240.09	0.03552984	-0.0382373 *
p937.63(a)(b)	0.0172166	-0.0181005 *	n73.09(a)	0.00525237	0.03761704 *
p273.18(b)	-0.01518347	0.01796049 *	n719.63(a)	0.02130188	0.03758092 *
p395.18	0.00668088	-0.01758732 *	n220.09(b)	-0.01280534	-0.0373915 *
p566.09	0.00377589	-0.01749029 *	n263.18	0.01804128	-0.03682512 *
p277.18	0.01683382	-0.017469 *	n146.09	-0.01841513	0.03644474 *
p145	-0.01454418	0.01744678 *	n326.18	-0.01679075	0.03586411 *
p211.09	0.01670983	-0.01742886 *	n293.27	-0.01212114	0.03555941 *
p114	-0.00871498	-0.01550419 *	n102.09	-0.01811975	0.03527974 *
p819.54(a)	-0.00343397	0.01544675 *	n148.09	-0.01425118	0.03515105 *
p633.36(a)	-0.01071156	0.01535235 *	n481.27(a)	0.01109477	0.03368782 *
p258.18	0.01142149	0.01517629 *	n267.18	0.00578409	0.03331052 *
p329.18	0.00718366	-0.01507546 *	n443.18(a)	0.00131349	0.03201525 *
p160	-0.01225392	-0.0144293 *	n337.09	-0.00948511	0.0313457 *

p183.09	0.0061735	0.01439279 *	n381.18	-0.02948856	0.0297257 *
p241.72	-0.00752764	0.01437226 *	n157	-0.01703734	0.0286304 *
p255.18	0.01406834	-0.01415898 *	n510(a)	-0.00357686	-0.02817446 *
p449.18	0.00995409	0.01402452 *	n100	-0.0243482	0.02771759 *
p175	-0.0032051	-0.01379232 *	n601.17(a)	0.02118683	-0.0271685 *
p237.09	-0.00127061	0.01374697 *	n139	-0.00950263	0.02701138 *
p113.45	-0.0007686	-0.0130095 *	n138	0.01237562	-0.02552221 *
p195.09	-0.00751466	-0.01291693 *	n125	0.0032058	-0.02465505 *
p391	0.00718986	0.01250418 *	n312.18	-0.0084258	0.02462516 *
p313.18	0.01017652	-0.01222449 *	n539.27(a)(b)	-0.01919355	-0.02183815 *
p312.08	0.0083926	0.01150936 *	n265.18	-0.00726031	0.01760863 *
p210	-0.01036481	0.01112708 *	n483.27(a)	-0.00560975	0.01661438 *
p231.09	0.01035481	-0.0110806 *	n165	-0.01307494	-0.01572853 *
p106	-0.01057506	-0.01094875 *	n199.09	0.00973433	0.01531145 *
p519.27	-0.00561853	0.01036538 *	n349.09	-0.01219379	0.01425127 *
p58.09(a)(b)	-0.00474148	0.01029416 *	n427.18(a)	-0.00271607	-0.01186634 *
p493.27	0.00805664	-0.01001674 *	n399.18(a)	0.00245719	-0.0076831 *

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**Table S12.** Statistics of 16 metabolites and significance tests of their mean values in *Brachypodium distachyon*, *B. stacei* and *B. hybridum*. N, number of ecotypic samples analysed. ANOVA (F; d.f. 2) test of variables used for comparisons among species. Superscripts denote Tukey pairwise comparisons between species; means with the same letter do not differ significantly ( $p < 0.05$ ).

Species	p139.9	p147.09	p182	p214.09	p235.09	p296.18	p381.18	p816.54	n135	n163	n179.09	n203.09	n281.18	n315.18	n346.09	n385.18
<b><i>B. distachyon</i></b>																
N	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75
Minimum	19200	992	7500	4210	9750	1990	212000	53800	1530	1850	817	20400	7880	7630	3210	7660
Maximum	61800	3370	67100	21600	45300	9660	689000	198000	5620	18800	3460	101000	40100	51700	22100	56000
Mean	36630,7 <sup>b</sup>	1920,7 <sup>b</sup>	23239,9 <sup>b</sup>	9694,1 <sup>c</sup>	21650 <sup>b</sup>	4980,5 <sup>c</sup>	403173,3 <sup>b</sup>	123758,7 <sup>b</sup>	3302,8 <sup>a</sup>	8027,1 <sup>a</sup>	2023,5 <sup>b</sup>	42169,3 <sup>b</sup>	18838,3 <sup>c</sup>	20624,4 <sup>c</sup>	10630,9 <sup>c</sup>	22654 <sup>a</sup>
Std. Deviation.	9309,1	575,1	11833,8	3591,3	6625,5	1572,3	90742,6	31246,4	946,3	2984,9	653,6	12740,1	6467,3	7370,2	3846,6	9717,9
<b><i>B. stacei</i></b>																
N	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45
Minimum	25400	1160	8900	6820	9680	3320	207000	104000	1530	5470	954	16200	29300	20400	7220	3110
Maximum	92100	4860	64700	20100	46100	21400	561000	231000	7940	28800	4200	66000	228000	84600	63800	15000
Mean	57448,9 <sup>a</sup>	2225,6 <sup>b</sup>	30130,2 <sup>a</sup>	13670,9 <sup>b</sup>	28952,9 <sup>a</sup>	11051,3 <sup>a</sup>	327288,9 <sup>c</sup>	162911,1 <sup>a</sup>	3556,4 <sup>a</sup>	17264 <sup>b</sup>	2352,3 <sup>a</sup>	27671,1 <sup>c</sup>	93328,9 <sup>a</sup>	46406,7 <sup>b</sup>	22729,3 <sup>b</sup>	6548 <sup>c</sup>
Std. Deviation.	14966,7	732,9	11627,2	3771,383	7632,292	3429,171	73826,462	35832,8	1451,9	5631	649,7	11277,3	37823,9	17138,8	10327,4	2676,4
<b><i>B. hybridum</i></b>																
N	59	59	59	59	59	59	59	59	59	59	59	59	59	59	59	59
Minimum	7570	1230	5630	6790	8270	2880	231000	77900	1980	1490	730	17400	20700	12600	7570	4580
Maximum	66200	5540	50800	30800	28900	10900	698000	257000	20800	21700	2260	102000	148000	151000	67100	25000
Mean	31426,6 <sup>c</sup>	2641,2 <sup>a</sup>	20013,9 <sup>b</sup>	16988,3 <sup>a</sup>	15325,4 <sup>c</sup>	5996,4 <sup>b</sup>	463932,2 <sup>a</sup>	175786,4 <sup>a</sup>	8053,2 <sup>b</sup>	8982,4 <sup>a</sup>	1342,7 <sup>c</sup>	55983 <sup>a</sup>	71940,7 <sup>b</sup>	59771,2 <sup>a</sup>	32733,2 <sup>a</sup>	11138,6 <sup>b</sup>
Std. Deviation.	11055,32	1021,6	9612,4	6091,799	4122,737	1570,903	129507,8	46207,8 <sup>a</sup>	4531,9	4020,2	408,3	18316,1	35929,7	26458	12993,3	4461,2
<b>F</b>	71,1	13,9	10,8	41,9	61,5	114,7	23	34,2	56	78	42,1	48,8	113	81,1	92,2	88,2
<b>p</b>	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.002	<0.003	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Fig. S1

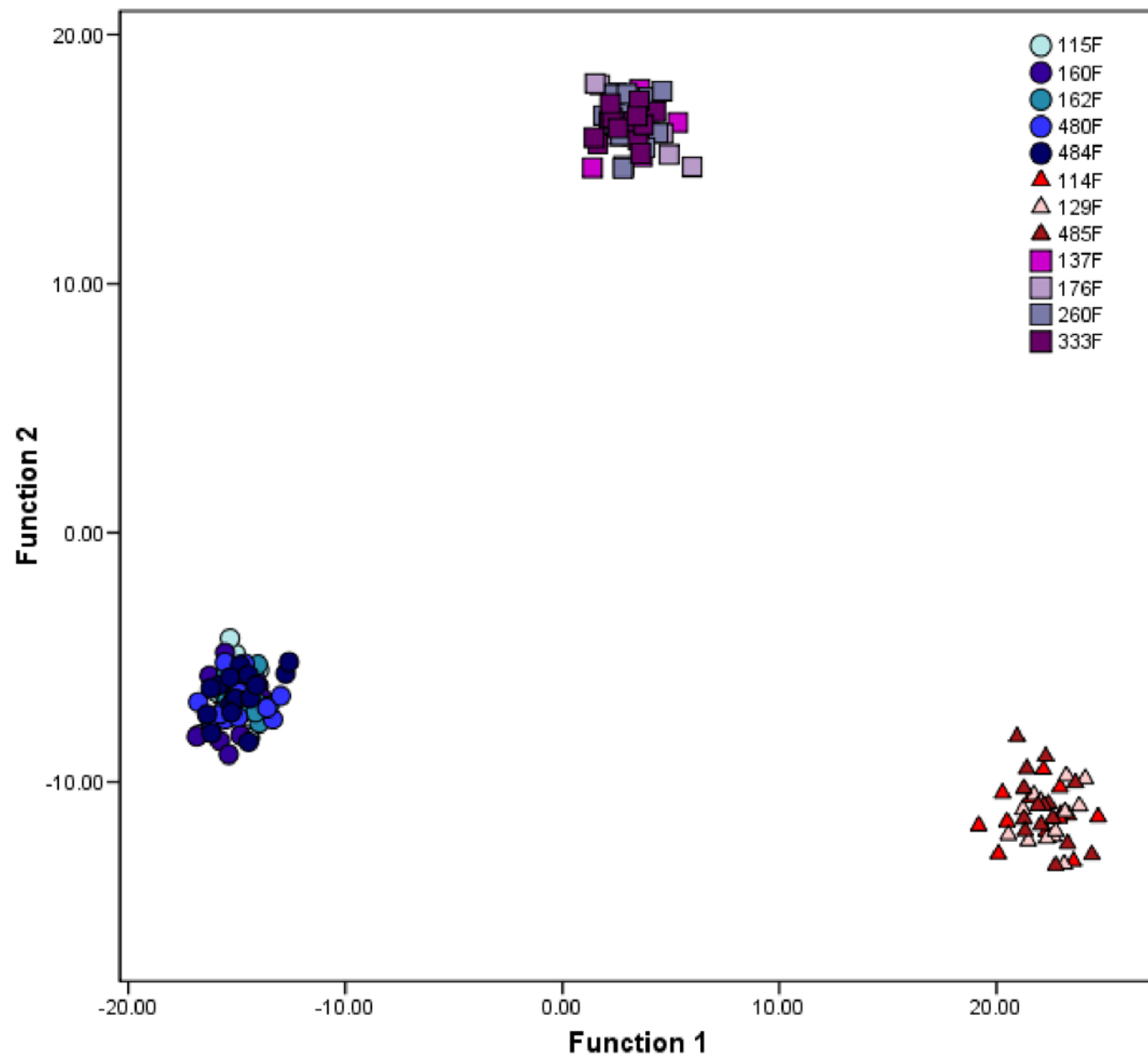
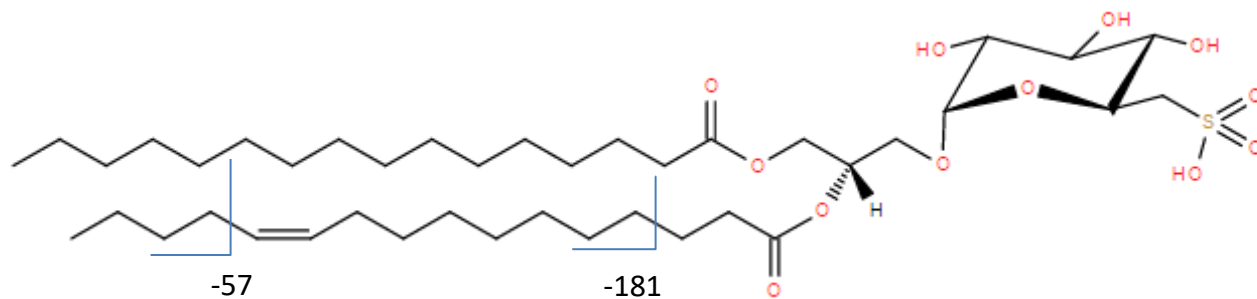


Fig. S2

$m/z$  815.54  $[M+Na]^+$  (SQDG)



$$815.54 - (C_4H_9) 57.27 = 758.27$$

$$815.54 - (C_{13}H_{25}) 181.36 = 634.18$$

Brachy-P-815 #1-30 RT: 0.00-0.33 AV: 30 NL: 2.28E2  
F: ITMS + p ESI Full ms2 815.54@cid30.00 [220.00-820.00]

